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This report is required by law (7 USC 2143) Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150

Set reverse side for additional information

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1 REGISTRATION NO. 14-R-0144/1749

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA include Zip Code)

Charles River Laboratories Inc.
251 Ballardvale Street
Wilmington, MA 01887 978-658-6000

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

See attached listing.

FACILITY LOCATIONS (Sites)

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, research, or surgery but not yet used for such purposes	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used	E. Number of animals upon which teaching experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in those animals and the reasons such drugs were not used must be attached to this report)	F. TOTAL NO OF ANIMALS (Cols. C + D + E)
4. Dogs	90	1728	902	18	2648
5. Cats	0	0	0	0	0
6. Guinea Pigs	299	3020	1344	0	4364
7. Hamsters	240	1162	4	24	1190
8. Rabbits	260	6059	1179	246	7484
9. Non-human Primates	3145	1288	2347	3	3638
10. Sheep	0	0	133	0	133
11. Pigs	21	117	512	9	638
12. Other Farm Animals					
Cows	0	0	2	0	2
Goats	0	0	4	0	4
13 Other Animals					
Cotton Rat	30	30	68	0	98
Gerbil	0	23	30	0	53
Degu	65	0	0	0	0

ASSURANCE STATEMENTS

- 1) Professionally acceptable standards governing the care, treatment, and use of animals including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility
- 2) Each principal investigator has considered alternatives to painful procedures.
- 3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.
- 4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional Official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

(B)(6) (B)(7)(C)

DATE SIGNED

11/21/2006

APHIS FORM 7023

(Replaces VS FORM 18-23 (OCT 88) which is obsolete

(AUG 91)

Preface:

Animals placed in Column "E" in this report were utilized in Preclinical Services Facilities (listed above) belonging to Charles River Laboratories. These animals were enrolled in studies undertaken for product registration purposes based on regulatory guidelines of the FDA 21 CFR 312.23 for pharmacology and toxicology studies and the Red Book. Guidance for study design and conduct also conformed with recommendations by the International Conference on Harmonization Guidelines. This guidance includes Part VII, DHHS, FDA, International Conference on Harmonization; Guidance on non-clinical safety studies for the conduct of human clinical trial for pharmaceuticals, Federal Register, Vol. 62, #227, November 25, 1997.

Depending upon the nature of the compound, certain other regulations and guidelines promulgated by the FDA, EPA, TSCA, FIFRA and the OECD also apply and are listed in the Applicable Guidelines/Regulations section below.

Animals could be placed in Category "E" both prospectively and retrospectively. Retrospective diagnosis of pain or distress was made by the Attending Veterinarian in conjunction with the Study Director. In order to insure that under reporting did not occur, the magnitude and duration of clinical signs were considered in making the diagnosis of pain or distress as was the length of time between the development of clinical signs and actions such as discontinuation of compound administration, administration of therapeutic measures that may or may not have included the use of analgesics or euthanasia. Animals that were moribund or had any clinical signs of sufficient magnitude to warrant early termination from the study (moribund euthanasia) were included only if there was, in the opinion of the Attending Veterinarian, a delay in conducting euthanasia in order to acquire additional data or if there was a failure in treatment regimens to resolve the condition. Animals showing any clinical signs of acute toxicity, even though they were mild or were not specifically related to pain or distress, were included if the animal was maintained on study following the development of such signs for more than a reasonable period of time for the signs to resolve (usually less than 24 hours) without the initiation of treatment to relieve the signs or to address the possible presence of pain or if the animal was not euthanized at that time. Any animals placed in Category "E" prospectively were assigned at the time of IACUC protocol review and were generally not re-categorized.

REVIEW OF CATEGORY "E" STUDIES

The following studies have been listed in Category "E" based upon the guidelines stated in the preface at the beginning of this report. Studies classified into Category "E" were conducted at Charles River Laboratories Preclinical Services located in Sparks, NV (site #05), Horsham, PA (Site # 014) and Redfield, AR (Site # 016) facilities. The study designs that resulted in certain animals being placed retrospectively into Category "E" were required by federal regulations and guidelines listed in the applicable regulations/guidelines section below. For the purpose of this report studies have been given a unique number that corresponds to the actual study number. For reasons of confidentiality, actual study numbers are not presented but are available to the USDA for on-site inspection or report follow-up. Category "E" explanations/details are listed separately for each study at each of the two sites.

CHARLES RIVER LABS PRECLINICAL SERVICES– (Horsham, PA - 014)

Study: #1

Animals: 11 Rabbits (high-dose group)

Type of Study: Oral (Stomach Tube) Reverse osmosis membrane processed deionized water Carboxymethylcellulose and Corn Oil and Intravenous Saline Developmental Toxicity Study in Rabbits

Guidelines/Regulations:

- The purpose of this study was to evaluate this rabbit strain from a new rabbit supplier and to collect historical control data for this strain. This study evaluates ICH harmonized Tripartite Guideline stages C and D of the reproductive process in a nonrodent species.

Diagnosis: 11 rabbits from this study have been placed in category E based on the presence of clinical/necropsy signs that can be presumed to have associated pain/distress and/or levels of body weight loss that can be presumed to have associated pain/distress. This study was this facility's own, designed to evaluate time mated New Zealand White rabbits from a new supplier. The approval states that no more than momentary pain/distress was expected. The rabbits arrived on various dates from December 2005 to April 2006. More than sixty veterinary exams were performed during the conduct of this study.

1 rabbit #5142 (III; corn oil by stomach tube) was euthanized per veterinary recommendation on gestation day 13. This rabbit lost 18.8% body weight from GD3 to euthanasia. This animal ate well during the acclimation period (GD3-GD6) but had low feed intake from GD 7 (first day of dosage) to euthanasia. All rabbits in this group received 5 ml/kg of corn oil on GD7 and GD8 then received a holiday (were not dosed) on GD9. This was done as a result of the soft feces observed and attributed to the dosage level of the corn oil. When dosage administration resumed on GD 10, the dosage was lowered to 2 ml/kg/day. All animals in this group were evaluated by veterinary staff for weight loss, feed intake and behavior on GD11 and GD12 and all received timothy cubes daily. At vet exam on GD13, this rabbit was noted as having apprehensive behavior and labored breathing. Necropsy evaluation showed an accumulation of hair in the stomach and ten live fetuses.

1 rabbit #5158 (III; corn oil by stomach tube) was found dead on GD11. This rabbit had fair feed intake during acclimation and as a consequence received two water sources, timothy cubes daily and feed offerings at two locations within the cage. Feed intake was low GD7-GD10. This rabbit lost 19% body weight GDO-GD10. Adverse clinical signs included soft feces and ungroomed coat. All rabbits in this group received 5 ml/kg of corn oil on GD7 and GD8 then received a holiday (were not dosed) on GD9.

This was done as a result of the soft feces observed and attributed to the dosage level of the corn oil. When dosage administration resumed on GD 10, the dosage was lowered to 2 ml/kg/day. All animals in this group were evaluated by veterinary staff for weight loss, feed intake and behavior on GD11 and GD12 and all received timothy cubes daily. Necropsy revealed a perforation in the right bronchus and stomach erosions. The technician dosing this rabbit was experienced in this dosage procedure and had no history of problems with this procedure in rabbits. Incidents of this nature are tracked by lab management in order to identify patterns which may indicate the need for re-training of a member of the laboratory staff.

1 rabbit #5159 (III; corn oil by stomach tube) was euthanized on GD11 per veterinary recommendation due to the magnitude of acute body weight loss. The rabbit remained alert and active. The only adverse sign noted was soft feces. This rabbit lost 23.7 % body weight from GD3-GD11 and feed intake was low during this same period. All rabbits in this group received 5 ml/kg of corn oil on GD7 and GD8 then received a holiday (were not dosed) on GD9. This was done as a result of the soft feces observed and attributed to the dosage level of the corn oil. When dosage administration resumed on GD10, the dosage was lowered to 2 ml/kg/day. At necropsy, this rabbit appeared normal and nine live fetuses were present.

1 rabbit #5161 (IV; intravenous saline bolus) was found dead at morning viability checks on GD26. This rabbit lost 13% body weight from GDO-GD8, maintained weight from GD8-GD12 and lost 15% from GD12-GD25. Feed intake was zero during acclimation and consequently two water sources, timothy cubes and feed offerings at two locations within the cage were provided. All rabbits in this group were monitored on GD13 and GD17-GD20 for feed and body weight changes as well as for general condition. The only adverse sign noted for this rabbit was scant fecal output. At necropsy, fetal tissue was found in the rabbit's stomach indicating abortion occurred prior to death.

1 rabbit #5178 (IV; intravenous saline bolus) aborted and was euthanized on GD23. This rabbit lost 20% body weight from GDO to GD22. Feed intake was zero during acclimation so two water sources, timothy cubes and feed at two locations within the cage were provided. Feed intake remained low from GD3-GD22. All rabbits in this group were monitored on GD 13 and GD 17 -GD20 for feed and body weight changes as well as for general condition. No adverse clinical signs were present in this rabbit. This rabbit appeared normal at necropsy exam with evidence of fetal tissue present in the stomach. The ten aborted fetuses were dead.

1 rabbit #5181 (V; corn oil by stomach tube) aborted and was euthanized on GD28. This rabbit lost 15% body weight from GD8 to GD28. Feed intake was good until GD9, low from GD9-GD19, good from GD19-GD20 then low from GD20-GD28. No adverse clinical signs were present in this rabbit. Rabbits in this group were evaluated by the vet staff for feed and body weight changes and general condition on GD4-GD7 and weekly thereafter. This rabbit was normal at necropsy and three live fetuses and one late resorption were present.

1 rabbit #5185 (V; corn oil by stomach tube) was found dead on GD14. This rabbit lost 21% body weight from GDO to GD13. Feed intake was low every day except for GD 4 when intake was good and on GD6 when intake was fair. (Feed intake level was inadvertently not recorded on GD5.) This rabbit received two water sources, timothy cubes and two feed locations within the cage as a result of low feed intake at arrival. On GD9, a veterinary exam was done due to the low feed intake and rabbit was otherwise normal. Daily grooming to minimize ingestion of loose hair while feed intake was low was initiated. At examination on GD 11, there was no additional weight loss and activity level was noted as perhaps reduced. No other adverse signs were noted for this rabbit except for soft feces on GD9. The rabbit was normal at necropsy exam and ten dead fetuses were present.

1 rabbit #5189 (V; corn oil by stomach tube) was euthanized on GD14 per veterinary examination

based on the magnitude of body weight loss. (Note: As a result of the body weight losses observed in some rabbits received in the initial months of this study, the study director approved euthanasia of any rabbit during the remaining portion of the study with a weight loss of 21% or more.) This rabbit lost 21% body weight from GDO-GD13. Feed intake was low during this same period except for GD8 when intake level was moderate. (Feed intake was inadvertently not recorded on GD5.) This rabbit received two water sources, timothy cubes and two feed locations within the cage as a result of low feed intake at arrival. Exams were conducted on GD4-GD7 and GD14. No adverse signs were noted in this rabbit and rabbit remained bright, alert and inquisitive. This rabbit was normal at necropsy exam and six resorbed fetuses were present.

1 rabbit #6132 (VI; intravenous saline at 0.66 ml/min) died during morning viability checks on GD29 just minutes after dehydration and decreased motor activity were first noted. This rabbit lost 7.8% body weight from GDO-GD3, gained 11 % body weight from GD3-GD16 and lost 12% weight from GD16-GD29. Feed intake was good during acclimation through GD17 then low from GD17-GD29. Adverse signs noted prior to the day of death included scant fecal output and ungroomed coat. This group was examined weekly by the vet staff for feed intake, weight loss and general condition. Necropsy exam showed a pale liver, a distended gallbladder, friable fat and 11 dead fetuses.

1 rabbit #6169 (VII; group not dosed) was euthanized on GD23 for evidence of impending abortion and due to the magnitude of weight loss per veterinary recommendation. This rabbit lost 11.7% body weight from GDO-GD9, maintained weight from GD9-GD12, lost 3.6% from GD12-GD15, maintained weight from GD15-GD19, lost 1.0% from GD19 GD20 and maintained weight from GD20-GD23. Feed intake was low GD3-4, GD6-23 and good on GD5. This rabbit received two water sources, timothy cubes and two feed locations within the cage as a result of low intake on GD3. Scant feces on GD12-GD14 were noted as the only adverse sign in this rabbit prior to the day of euthanasia. On GD21, veterinary exam indicated rabbit was normal on physical exam and in behavior, was eating the timothy cube daily, had two water sources and was offered feed at two locations within the cage. At re-exam on GD23, rabbit remained bright, alert and inquisitive. There was evidence of impending abortion and based on the magnitude of weight loss, this rabbit was euthanized. This rabbit was normal on necropsy exam. Ten live fetuses and three late resorptions were present.

1 rabbit #6189 (VIII; group not dosed) aborted and was euthanized on GD27. A scab (2.0 x 0.5 cm) on the right rear leg was noted at arrival examination and was no longer present (scab was healed) on GD7, the first day of dosage. While no pain/distress was noted regarding the scab at arrival, it can be presumed that the changes in the skin resulting in a scab of this size may have been associated with pain/distress at some point in time. This rabbit lost 14% body weight from GD12-26. Feed intake was good from acclimation to GD13, fair from GD13-GD16, low from GD16-GD18, fair from GD18-GD20, not recorded on GD21-GD22 and low from GD22-GD26. Necropsy showed a pale liver. All fetuses were dead or resorbed. The reason for classifying as E is the weight loss, not the skin wound that occurred prior to receipt at the facility and resolved without complications.

Study: #2

Animals: 1 Rabbit

Type of Study: Intravenous Dose Range - Finding Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range

study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 1 rabbit from this dosage range-finding study conducted in July-August 2006 has been placed in category E as described in the paragraph below. The study rabbits were dosed intravenously by slow bolus injection once daily from gestation day 7 through 19. The dosage levels were 0,5, 15,30 and 60 mg/kg/day. The test article, a drug acting on blood or blood-forming organs, caused swelling and excessive bleeding at the injection site in rats. It had not been administered to rabbits before. The approved humane endpoint for addressing rabbits in pain/distress was for the study director and vet staff to decide at the time of occurrence. Slight vaginal bleeding was approved to not be considered an abortion for this study. All rabbits on study were examined twelve times, rabbits in the highest dosage groups (IV and V) were examined thirteen times and twelve individual rabbit exams were completed during the 28 day duration of this study.

1 rabbit #7991 The animal was diagnosed with pain/distress due to an increased respiratory rate during five veterinary exams. During one of these exams, a reduction in the level of inquisitiveness was also noted before returning to normal at exam the following day. Bloody urine was also noted from gestation day 14 through 23. This rabbit remained bright, alert and responsive throughout the study and ate well every day of the study. An overall body weight gain of 23.3% occurred from gestation day 0 to 29 with only four non-consecutive, one-day minor loss (less than 5%) intervals. This rabbit was examined on twenty-one occasions either individually or during monitoring of the highest dosage groups. This rabbit was euthanized according to the study schedule on gestation day 29. The rabbit was normal at necropsy examine and was not pregnant. This rabbit has been placed in category E because an increased respiratory rate for ten days and a reduction in inquisitiveness can be presumed to have been associated with pain/distress.

Study: #3

Animals: 1 Rabbit (mid-dose group)

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, march 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 1 rabbit from this developmental toxicity study has been placed in category E as described in the paragraph below. This study was conducted in February-March 2006. This study was approved to cause pain/distress from test article induced body weight loss in groups IV and V. The approval states that animal pain and distress can not be relieved. The approval states that the plan for addressing animal with pain/distress is for the study director and veterinary staff to decide at the time of occurrence.

1 rabbit #5864 (group IV) was normal on arrival at the facility and normal prior to assignment to study. On the first day of dosage, the animal began to loose weight and had reduced feed intake. At veterinary exam, the rabbit had lost 9% body weight in five days, had loose feces and remained active, alert and inquisitive. As a result, treatment was initiated. An alfalfa cube was provided daily to encourage improved feed intake and daily brushing was initiated to remove loose hair in order to minimize the

ingestion of hair during self-grooming. On re-exam two days later, the rabbit had lost an additional 6% body weight, remained alert though appeared somewhat apprehensive and subjectively, less active. Treatment (the alfalfa and grooming) was continued. The following morning on re-exam, the rabbit had lost an additional 5% body weight and though alert was not active or inquisitive. This rabbit was euthanized at the veterinarian's request that same morning. This rabbit has been placed in category E because the apprehension and reduced activity level that began on the day prior to the animal's euthanasia can be presumed to be associated with pain/distress.

Study: #4

Animals: 9 Rabbits

Type of Study: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 4 rabbits from Part A of this dosage range-finding developmental toxicity study conducted in May 2006 have been placed in category E for the reasons described in the paragraphs below. In Part A, non-mated rabbits were dosed by stomach tube once daily for five days then euthanized on the day after the last dosage administration. Dosage levels were 25, 100, 200 and 400 mg/kg/day. The expected test article effect was a reduction in body weight gain. The study was approved with moribund condition as a humane endpoint. The study was approved allowing the provision of timothy cubes to rabbits with low feed intake and weight loss. Veterinary exams were conducted daily.

4 rabbits #6575 (200 MKD), **#6576** (200 MKD), **#6579** (400 MKD) and **#6580** (40 MKD) had low to no feed intake over the entire study and lost 17.8%, 15.7%, 22.8% and 16.9% body weight, respectively, over the 6 day study period. The only adverse clinical signs were soft and/or scant feces and ungroomed coats. Beginning on day 3 of the study, all rabbits in the 200 and 400 MKD level groups were provided with a timothy cube daily to offer something palatable to eat in addition to the pelleted feed provided daily. And beginning on day 4 of the study, the floor grates of the rabbits in these same groups were cleaned daily to remove the soft feces. These rabbits have been placed in category E because it can be presumed that pain/distress was associated with body weight loss of the magnitude that occurred in a period of six days.

5 rabbits in the highest dosage group from Part B of this dosage range-finding developmental toxicity study conducted in June 2006 have been placed in category E for the reasons described in the paragraphs below. In part B of this study, mated rabbits were dosed once daily by stomach tube on gestation day 7 through 19. The satellite rabbits were euthanized on gestation day 20 and the main study rabbits were euthanized on gestation day 29. Dosage levels were 0, 3, 10, 20 and 30 mg/kg day. Reduced body weight gain was the expected test article effect. The study was approved with moribund condition listed as the humane endpoint. Over 28 veterinary exams were completed during the conduct of this study.

1 rabbit #7697 (30 MKD) aborted and was euthanized on GD22. This rabbit lost 12.1% body weight

from GD7 to GD 18, maintained weight from GD 18 to GD20 and lost 4.2% weight from GD20 to GD21. Overall weight loss from GD7 to GD21 was 16.5%. Feed intake was fair to low from GD 11 to GD21. The only adverse sign was soft or scant feces. All rabbits in this group had two water sources available beginning on GD12 and were provided with a timothy cube daily beginning on GD 14. This rabbit was examined daily from GD12 to GD22 and remained bright, active, alert and inquisitive. This rabbit was normal at necropsy exam. All fetuses were resorbed. This rabbit has been placed in category E because pain/distress can be presumed to be associated with body weight loss of the magnitude that occurred prior to resorption and abortion.

1 rabbit #7698 (30 MKD) aborted and was euthanized on GD20. This rabbit lost 5.7% body weight from GD 10 to GD12, maintained weight GD12 to GD13 and lost 9.0% from GD13 to GD19. Overall weight loss was 14.0%. Feed intake was low to fair from GD11 to GD20. The only adverse sign was scant or no feces. All rabbits in this group had two water sources available beginning on GD12 and were provided with a timothy cube daily beginning on GD 14. This rabbit was examined daily from GD12 to GD20 and remained bright, active, alert and inquisitive. This rabbit was normal at necropsy exam and all fetuses were resorbed. This rabbit has been placed in category E because pain/distress can be presumed to be associated with body weight loss of the magnitude that occurred prior to resorption and abortion.

1 rabbit #7699 (30 MKD) showed signs of an impending abortion and was euthanized on GD20. This rabbit lost 14.0% body weight from GD9 to GD20. Feed intake was low from GD 11 to GD20. Scant to no feces was noted from GD13 to GD20. All rabbits in this group had two water sources available beginning on GD12 and were provided with a timothy cube daily beginning on GD 14. This rabbit was examined daily from GD12 to GD20 and remained bright, active, alert and inquisitive. Both kidneys were dark red at necropsy exam. All fetuses were resorbed. This rabbit has been placed in category E because pain/distress can be presumed to be associated with the adverse necropsy finding and with body weight loss of the magnitude that occurred prior to euthanasia.

1 rabbit #7540 (30 MKD) was euthanized as scheduled on GD20. This rabbit lost 15.2% body weight from GD7 to GD20 and had low feed intake from GD10 to GD20. Scant to no feces was noted from GD13 to GD20. All rabbits in this group had two water sources available beginning on GD12 and were provided with a timothy cube daily beginning on GD14. This rabbit was examined daily from GD12 to GD20 and remained bright, active, alert and inquisitive until the morning of scheduled euthanasia on GD20 when apprehension was noted. This rabbit has been placed in category E because pain/distress can be presumed to be associated with apprehensive behavior and with weight loss of the magnitude that occurred prior to euthanasia.

1 rabbit #7542 (30 MKD) was euthanized as scheduled on GD20. 20.9% body weight was lost from GD7 to GD20. Feed intake was fair from GD7 to GD 11 and low from GD 11 to GD20. Adverse signs noted were scant or no feces from GD13 to GD20. All rabbits in this group had two water sources available beginning on GD12 and were provided with a timothy cube daily beginning on GD14. This rabbit was examined daily from GD12 to GD20 and was bright, alert and responsive at all of these exams though quiet, timid behavior was noted on GD17 and GD18. Beginning on GD17, this rabbit's daily feed ration was divided in half and offered at the front and at the back of the cage to help improve interest in eating. Inquisitiveness returned to normal on GD 19. This rabbit has been placed in category E because pain/distress can be presumed to be associated with timid behavior of a two-day duration and with weight loss of the magnitude that occurred prior to euthanasia.

Animals: 6 Rabbits

Type of Study: Intravenous Dosage-Range Finding Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: All 6 rabbits from Part A of this dosage-range finding study conducted in July 2006 have been placed in category E for the nature of and duration of adverse clinical signs including body weight loss and low feed intake. Non-mated rabbits were dosed once by one hour intravenous infusion. Group I received vehicle which contained 20% ethanol, group II received test article at 15 mg/kg and group III received test article at 50 mg/kg. Post dosage checks were done at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours and at the end of the day. The study was approved to cause no more than momentary pain/distress from test article effects. Vet exams were conducted three times on the day of dosage and daily thereafter. Part B of this study was not conducted.

2 rabbits #1498 (50 mg/kg) and **#1499** (50 mg/kg) were diagnosed with pain/distress during veterinary exams conducted one hour apart (at 1435 and at 1530) following dosage administration a short time earlier. Adverse signs that persisted for 1 to 2 hours included rapid respiration, red eyes, perinasal substance, twitches (#1498 only), head tilt (#1498 only). Decreased motor activity was present for approximately 2.5 hours then animals became increasingly more active and responsive by 4 hours after dosage. Red substance (urine) was observed soon after dosage and persisted until the following day. No pain/distress was diagnosed at the veterinary exam conducted at 1740. A second water source was provided on the day of dosage. No feed was consumed overnight and weight loss over 24 hours was 6.5%-8.4%. At exam just prior to scheduled euthanasia on the day following dosage, these rabbits were alert and responsive though less active than a normal rabbit would be.

2 rabbits #1496 (15 mg/kg) and **#1497** (15 mg/kg) were diagnosed with pain/distress during veterinary exams conducted one hour apart (at 1435 and at 1530) following dosage administration a short time earlier. Adverse signs observed after dosage included red eyes, head tilt (#1497 only), soft feces (#1497 only) and decreased motor activity. Decreased motor activity was present for approximately 2.5 hours then animals became increasingly more active and responsive by 4 hours after dosage. Red substance (urine) was observed soon after dosage and persisted until the following day. No pain/distress was diagnosed at the veterinary exam conducted at 1740. A second water source was provided on the day of dosage. No feed was consumed overnight. At the morning exam on the day after dosage, these rabbits were alert and responsive though less active than a normal rabbit would be. A dry timothy cube and a wet timothy cube were provided to each rabbit. An amendment was approved to maintain these rabbits on study (euthanasia was scheduled for the day after dosage by the protocol) so they could be dosed again in one week. Pain/distress was diagnosed during veterinary exams conducted daily for the next three days for low to no feed intake including timothy cubes, weight loss of 8.7% to 10.5% over five days, scant fecal output and for reduced activity level while alert and responsive. Four days following the initial dosage, these rabbits were determined to be unsuitable for continuation on study and were euthanized.

2 rabbits #1494 (vehicle) and **#1495** (vehicle) were diagnosed with pain/distress during veterinary exams conducted one hour apart (at 1435 and at 1530) following dosage administration a short time earlier. Decreased motor activity was present for approximately 2.5 hours then animals became increasingly more active and responsive by 4 hours after dosage. Red substance (urine) was observed

soon after dosage and persisted until the following day. No pain/distress was diagnosed at the veterinary exam conducted at 1740. A second water source was provided on the day of dosage. No feed was consumed overnight. At the morning exam on the day after dosage, these rabbits were alert and responsive though less active than a normal rabbit would be. A dry timothy cube and a wet timothy cube were provided to each rabbit. An amendment was approved to maintain these rabbits on study (euthanasia was scheduled for the day after dosage by the protocol) so they could be dosed again in one week. Pain/distress was diagnosed during veterinary exams conducted daily for the next three days for low to no feed intake including timothy cubes, weight loss of 9.3% to 13.9% over five days, scant fecal output, soft feces and for reduced activity level while alert and responsive. Four days following the initial dosage, these rabbits were determined to be unsuitable for continuation on study and were euthanized.

Study: #6

Animals: 11 Rabbits

Type of Study: Subcutaneous Dosage-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 11 rabbits from this dosage range-finding study conducted in May-June 2006 have been placed in category E based on the presence of adverse signs at one or more dosage sites of a nature that can be presumed to be associated with pain/distress. The rabbits were dosed subcutaneously once daily from gestation day 7 through gestation day 19. Six separate dosage sites were used and the sites were rotated daily. The study was approved to cause pain/distress from irritation by the test article at the injection site. The approval states that animal pain and distress can not be relieved. The approval states that a plan for animal experiencing pain/distress will be determined by the study director and veterinary staff at the time of occurrence. Clinical observations were recorded twice daily. These rabbits were monitored closely by the veterinary staff resulting in the euthanasia of two rabbits prior to the end of the study.

6 rabbits #7508 (II; 2 MKD), #7509 (II; 2 MKD), #7511 (II; 2 MKD), #7520 (IV; 10 MKD), #7522 (IV; 10 MKD), and #7530 (V; 20 MKD) had scabs at one or more injection sites ranging in size from 0.5 cm in diameter to 5.0 x 2.0 cm and ranging in duration from one day to fifteen days. No pain or distress was evident in response to manipulation and handling of these dosage sites. No rabbit lost body weight (except a minor loss of 10% over six days after the dosage period ended in rabbit #7520) and all ate well during the entire study. These rabbits were euthanized at the end of the study. These rabbits have been placed in category E because it can be presumed that the inflammatory process resulting in formation of scabs of the size noted was associated with pain/distress at some point during formation.

4 rabbits #7518 (III; 5 MKD), #7527 (V; 20 MKD), #7528 (V; 20 MKD) and #7529 (V; 20 MKD) had ulcerations in addition to scabs at one or more injection sites. The ulcerations ranged in size from 4.0 x 1.0 cm to 5.0 x 3.0 cm and ranged in duration from one day to three days. One dosage site on rabbit #7518 was treated by application of a cold compress for ten minutes daily for five days to resolve slight swelling at that site. No rabbit lost more than 1 % body weight at any daily weigh interval during the study and there was no overall body weight loss from study start to study end. All rabbits ate well

during the entire study except for a slight to moderate drop in feed intake from GD7 to GD17 in #7527. No pain or distress was evident in response to manipulation and handling of these dosage sites except for rabbit #7528 euthanized on the same day that discomfort to touch was first noted. All other rabbits were euthanized at the end of the study except for rabbit #7527 euthanized on the day that erythema and edema were first noted in association with a previously present ulcer; there was no discomfort associated with handling of the affected sites in this rabbit. These rabbits have been placed in category E because it can be presumed that the inflammatory process resulting in ulcer and scab formation of the sizes noted was associated with pain/distress at some point during the formation.

1 rabbit #7525 (V; 20 MKD) had swelling noted at one dosage site for one day and had no other adverse clinical signs during the study. This rabbit lost no body weight and ate well during the entire study. This rabbit was euthanized at the end of the study. This rabbit has been placed in category E because it can be presumed that the inflammatory process associated with the swelling in this rabbit caused pain/distress as some point.

Study: #7

Animals: 6 Rabbits

Type of Study: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 6 rabbits from this dosage range-finding study conducted in July-August 2006 have been placed in category E as described in the paragraphs below. Non-mated female rabbits were dosed once daily by stomach tube for five days and euthanasia was scheduled for the day after the last dosage. The dosage levels were 50, 100, 200 and 450 mg/kg/day. Timothy cubes were provided daily to offer a palatable food source in addition to the diet already being provided. Veterinary exams were conducted daily before dosage and at the end of the day. Study staff conducted checks each morning, hourly for four hours after each daily dosage and at the end of the day.

1 rabbit #8110 (450 MKG) was euthanized on day 3 of the study when the rabbit reached moribund condition. There were no adverse clinical signs present on days 1 and 2 of the study. Feed intake was low beginning on study day 1 and 13% body weight was lost from day 1 to day3. Adverse signs were first noted at the morning viability check on study day 3. At veterinary exam at 0850 on day 3, the rabbit was aware and able to rest comfortably but was not completely alert, was reluctant to move and unsteady on its feet. To date, no other rabbits had shown any adverse signs. This rabbit was monitored continually by veterinary staff for the next two hours and when the rabbit's condition became moribund, the rabbit was promptly euthanized. Pale kidneys were found at necropsy. This rabbit has been placed in category E because adverse signs that can be presumed to be associated with pain/distress were present for more than a momentary period of time (a minimum of 2.5 hours) prior to the onset of moribund condition and euthanasia.

1 rabbit #8107 (200 MKD) was found dead at the morning viability check on day 4 of the study. No adverse clinical signs other than ungroomed coat were present prior being found dead. 8.6% body

weight was lost from study day 1 to 4 and feed intake was low during this same time. At necropsy, the thymus was mottled red and one lung lobe was dark red. This rabbit has been placed in category E acknowledging that it is possible that adverse signs associated with pain/distress were present for more than a momentary period of time in the overnight hours prior to death.

1 rabbit #8108 (200 MKD) was euthanized on day 4 of the study by veterinary recommendation due to moribund condition. On study day 3 and at the time of dosage on day 4, scant fecal output was noted. At the one and two hour after dosage checks at 1013 and 1114 on day 4, head tilt, impaired ability to right and decreased activity were noted. In the afternoon at 1527, these same adverse signs were present and the rabbit was euthanized. No gross abnormalities were observed at necropsy. This rabbit has been placed in category E because adverse signs that can be presumed to be associated with pain/distress were present for more than a momentary period of time (approximately 5 hours) prior to the onset of moribund condition and euthanasia.

1 rabbit #8104 (100 MKD) lost 14% body weight from day of study 1 to day of study 6. Feed intake was reduced to a moderate level on study day 1-2 then was zero on study days 3-6. Adverse signs included scant feces and urine-stained abdominal fur on study days 4-6. This rabbit was euthanized as scheduled on day 6 of the study. This rabbit has been placed in category E because it can be presumed that pain/distress is associated with anorexia and with body weight loss of the magnitude experienced in six days.

1 rabbit #8105 (100 MKD) was euthanized by veterinary recommendation for moribund condition present at the morning viability check on day 5 of the study. The only adverse clinical sign present prior to day 5 was ungroomed coat on study day 4. 6.8% body weight was lost from study day 1 to study day 5. Feed intake was good on study day 1 to 2 then reduced by 50% on study day 3. Adverse signs present on study day 5 included ptosis, decreased activity, lacrimation, cold to touch, rales and perivaginal substance. Necropsy exam showed large kidneys, dark red lungs, red areas on the thymus and the liver mottled red and tan. This rabbit has been placed in category E acknowledging that it is possible that adverse signs associated with pain/distress were present for more than a momentary period of time in the overnight hours prior discovery and euthanasia.

1 rabbit #8106 (100 MKD) was found dead at the time of the morning viability check on study day 6. This rabbit maintained body weight during the study and had good daily feed intake values. No adverse signs were observed. There were no gross abnormalities present at necropsy. No rabbits in the 200 and 450 MKD groups survived to scheduled euthanasia and one rabbit in the 100 MKD group was also euthanized for similar adverse signs. This rabbit has been placed in category E acknowledging that the death was likely the cause of drug toxicity that was likely accompanied had adverse signs associated with pain/distress at some time in the overnight hours prior to death.

Study: #8

Animals: 2 Rabbits (control group)

Type of Study: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits
Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be

needed for the full study.

Diagnosis: 2 rabbits from part B of this dosage range-finding study conducted in September 2006 have been placed in category E as described in the paragraphs below. Mated rabbits were dosed once daily by stomach tube from gestation day 7 through 19 at dosage levels of 0, 10, 20, 40 and 50 mg/kg/day. Levels as low as 100 mg/kg/day given daily for five days resulted in adverse signs and unscheduled deaths and euthanasia in Part A of this study.

1 rabbit #8118 (10 MKD) died approximately four minutes after dosage administration on gestation day 16. Adverse signs present at the time of death were rales, hyperpnea and convulsions. Scant fecal output was noted the day prior to death. 7.4% body weight was lost from gestation day 13 until death. Feed intake decreased to moderate on gestation day 12, intake was low on gestation days 13-15 and intake was not recorded on the day of death. Necropsy exam showed spongy and mottled lung lobes, a mottled liver and tear in one lung lobe. This rabbit has been placed in category E because it is possible that lung injury and/or liver pathology associated with pain/distress was present for several days prior to death based on the body weight reduction, feed intake reduction and necropsy data. Lab management maintains a tracking system of accidental injuries caused by dosage administration as a way to identify any staff member that may be experiencing problems with dosage technique.

1 rabbit #8134 (50 MKD) lost 11.5% body weight from gestation day 17 to 21 then gained body weight daily until scheduled euthanasia. Feed intake was reduced to a moderate level from gestation day 7-12, to a fair level from gestation day 12-14, to a low level from gestation day 14-20, to a moderate level from gestation day 20-22. Intake was good from gestation day 22 until the end of the study. This animal has been placed in category E acknowledging the likelihood that pain/distress from drug toxicity was more than slight and more than momentary because it resulted in low feed intake (0-5 grams per day) in a pregnant animal for six days.

Study: #9

Animals: 1 Rabbit (control group)

Type of Study: Oral (Stomach Tube) Bridging Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 1 Rabbit #2682 (control group) was normal for the entire study until adverse signs were observed at the time of death on 10/1/05, gestation day 24. The rabbit gained body weight daily and ate well every day during the entire study. The dosing period ended on gestation day 19. This rabbit was normal at the time of the morning viability check on 10/1/05. In the afternoon, this rabbit was noted with decreased motor activity and an abdominal mass which based on the necropsy observations, is presumed to be the stomach. The rabbit died at the same time the adverse signs were first discovered before any intervention as possible. At necropsy, all fetuses were resorbed, the stomach was distended with gas and the stomach mucosa was eroded. Because there is no way of determining the exact time of onset or the duration of the adverse clinical signs, this rabbit has been placed in category E acknowledging that more than momentary pain and distress may have occurred prior to death.

Study: #10**Animals: 1 Rabbit** (high-dose group)**Type of Study:** Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits**Guidelines/Regulations:**

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 1 rabbit in the highest dosage group (50 mg/kg/day) from this stomach tube developmental toxicity study has been placed assigned to category E. This study was conducted in December 2005 through January 2006. The study was approved with the knowledge that pain/distress could occur from body weight loss and low feed intake as expected test article effects at the high dose level. The approval states that animal pain and distress can not be relieved. The approval states that the plan for addressing rabbits in pain/distress is for the study director and veterinary staff to decide at the time of occurrence.

1 rabbit #4591 (50 MKD) lost body weight, consumed very small amounts of feed daily and had reduced water consumption from the beginning of dosage administration (gestation day 7) until euthanasia following premature delivery on gestation day 28. At veterinary exam on 1/4/06, this rabbit has lost 1.4 % body weight over 24 hours in association with four days of low feed intake. The rabbit was otherwise normal on exam. (Note: The exam on 1/4/06 inadvertently failed to note that the animal had a cumulative weight loss of 19% to date.) Reduced feed intake and weight loss was expected at this dosage level. The dosing period had just ended so improvement was expected. At re-exam on 1/6/06, an additional 2% body weight was lost and feed intake remained low. An alfalfa hay cube was provided daily to encourage improved feed intake. On 1/10/06 exam, an additional 3% body weight loss was noted and feed intake including the alfalfa cube, remained low. The rabbit was well-groomed and bright, alert and active. Euthanasia was scheduled for gestation day 29 on 1/13/06. On 1/12/06, this rabbit delivered 3 dead fetuses and several resorptions and was euthanized at that time. The only adverse observation at necropsy was a small spleen. Total weight loss was 25%. This rabbit has been placed in category E because the duration of low feed intake and the magnitude of the weight loss can be presumed to be associated with pain/distress.

Study: #11**Animals: 4 Rabbits****Type of Study:** Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits**Guidelines/Regulations:**

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 4 rabbits from this dosage range-finding developmental toxicity study conducted in

August-September 2006 have been placed in category E as described in the paragraphs below. The mated rabbits were dosed once daily by stomach tube at levels of 0, 750 and 1,500 mg/kg/day on gestation days 7 through 19. On the first two days of dosage, clinical observations were done at 2 and 4 hours after dosage and at the end of the day. On the remainder of the dosage days, clinical observations were done at 2 hours after dosage. The test article had not been given to rabbits before. The approval indicates that maternal toxicity was not expected and that rats given similar dosages showed no adverse effects. When a rabbit in the 1500 MKD was euthanized on gestation day 8 due to adverse clinical signs, an addendum to the approval was written. This provided guidance about what adverse signs would necessitate euthanasia and what signs would result in monitoring for additional adverse effects.

3 rabbits #8141 (0 MKD), **#8143** (750 MKD) and **#8145** (750 MKD) had one or more periods (range of duration was 5 to 7 days) of low daily feed consumption (0-10 grams) during or at the end of the dosage period. Body weight loss during these periods ranged from 4.5-9.3%. Soft and/or scant feces were also observed. No other adverse signs were present. These rabbits have been placed in category E acknowledging that low feed consumption for the periods of time experienced in these pregnant rabbits is indicative of more than momentary pain/distress.

1 rabbit #8148 (1500 MKD) lost 10.3% body weight from gestation day 12-21 and then gained 11.2 % from gestation day 22 to 29 (scheduled euthanasia.) Feed consumption was reduced to moderate from gestation day 7-14, to low from gestation day 14-20, to fair/moderate from gestation day 20-22 and was good from gestation day 22-29. Fecal output was soft and/or scant during the period of low feed consumption. Vaginal mucous membranes were edematous on gestation day 14-16 then inflamed on gestation day 16. The inflammation decreased and the area was normal by gestation day 19. A timothy cube was provided daily beginning on gestation day 16 and was consumed daily by the rabbit. When the dosage period ended on gestation day 19, the rabbit began gaining weight daily and feed consumption greatly improved. Daily timothy cubes were discontinued on gestation day 24. This rabbit has been placed in category E acknowledging that low feed consumption for the period of time experienced in this pregnant rabbits is indicative of more than momentary pain/distress. The vaginal swelling and inflammation can also be presumed to be associated with more than momentary pain/distress.

Study: #12

Animals: 2 Rabbits

Type of Study: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits, Including a Toxicokinetic Evaluation

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 2 rabbits from Part A of this dosage range-finding developmental toxicity study have been placed in category E as described in the paragraphs below. In Part A of this study, the test article was given once daily for five days by stomach tube followed by euthanasia on the day after the last dose. The test article was expected to have adverse effects on the central nervous system and mortality as an approved end point. Exams were conducted daily after dosage administration and on the last day of the study prior to scheduled euthanasia.

1 rabbit #6699 (60 mg/kg/day) was found dead at 7:24 a.m. on day 5 of the study. This rabbit was

normal in appearance and behavior at all prior exams including the exam conducted at 2:20 p.m. after dosage on the day before death. Feed intake was low to none from the first dosage until death and a total of 11.6% body weight was lost from first dosage until death. This rabbit was normal at necropsy exam(the test article was expected to affect the central nervous system so the absence of adverse gross exam observations is not unexpected). Note: Adverse clinical signs attributed to the test article toxicity were seen at levels as low as 10MKD so the dosages elected for Part B of this study were 0, 1, 3 and 6 MKD. This rabbit has been placed in category E acknowledging that more than momentary pain/distress may have occurred in the time between the last exam and the time of discovery of death attributed to test article toxicity..

1 rabbit #6700 (90 mg/kg/day) was found dead at 6:25 p.m. on day 5 of the study. At exam at 2:04 p.m. on day 5 of the study, this rabbit was noted as alert and responsive with a mildly sedated appearance. Feed intake was low to none from the first dosage until death and a total of 12% body weight was lost from first dosage (day of study) until death. This rabbit was normal at necropsy exam. (the test article was expected to affect the central nervous system so the absence of adverse gross exam observations is not unexpected). Note: Adverse clinical signs attributed to the test article toxicity were seen at levels as low as 10MKD so the dosages elected for Part B of this study were 0, 1, 3 and 6 MKD. This rabbit has been placed in category E acknowledging that more than momentary pain/distress may have occurred in the time between the last exam and the time of discovery of death attributed to test article toxicity.

Study: #13

Animals: 4 Rabbits

Type of Study: Intramuscular Repeat Dose Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Department of Health and Human Services Food and Drug Administration (CBER). Guidance for Industry: consideration for plasmid DNA vaccines for infectious disease indications. (Draft Guidance); February 2005.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

Diagnosis: 4 rabbits from this repeat dose toxicity study conducted in June-September 2006 have been placed in category E as described in the paragraphs below. Test article (a vaccine) or vehicle, was injected intramuscularly to the quadriceps muscle via a Biojector 2000 (0.5 ml per site, two sites spaced approximately 1 inch apart.) Injections were done on days 1, 22, 43 and 64 of the study and alternate rear legs were used for each date of injection. The approval indicates that it was not known whether or not the test article would result in toxicity and that any pain/distress from toxicity would not be relieved by anesthetics, analgesics or tranquilizers. The approval indicates that supportive care could be provided at the discretion of the veterinary staff and the study director. The approval indicates that the study did not contain any painful/distressful procedures. The animals were monitored at least twice daily by the study staff. The injection sites were observed daily by Draize scoring for one week after each injection. Veterinary staff monitored the animals at least weekly.

1 rabbit #7874 (4 mg, male) was found dead at the morning viability check on day 50 of the study. This rabbit ate 100% of the feed provided every day of the study and no body weight was lost. On day 46 of the study during routine veterinary monitoring, this rabbit was examined for mild swelling, warmth and purple discoloration on the right rear leg at the site of injection on day 43 of the study. The rabbit exhibited discomfort when the area was handled. The application of cold compresses to the area was recommended. Treatment was not approved by the study director this was acknowledged by the IACUC Chair. At re-exam on day 47 of the study, the site remained mildly swollen and purple but no discomfort

was apparent when the area was handled. At re-exam on day 48 of the study, the swelling and purple discoloration were no longer apparent. Necropsy showed bruising and discoloration of the muscle at the injection site. This animal has been placed in category E because the animal exhibited discomfort at the injection site that was likely present for more than a momentary length of time prior to discovery on day 46 of the study. The necropsy observations also support the fact that more than momentary pain/distress was experienced prior to death.

1 rabbit #7882 (vehicle, female) lost 16.5 % body weight from study day 1 to study day 8 and feed intake was low from study day 2 until study day 14. This rabbit was examined daily from study day 3 through study day 14 and bi-weekly until study day 23. This rabbit ate the timothy cube provided daily beginning on study day 3 and began to maintain and then gain body weight beginning on study day 11. Two timothy cubes were provided daily beginning on study day 5. On study day 8, soft feces was observed and the cage floor was cleaned twice daily to remove any accumulation of feces. On study days 10-12, the rabbit was less inquisitive than other rabbits and this improved to normal on study day 13. On study day 12, the feed ration was divided into two portions and offered at two locations inside the cage. The lost body weight was completely re-gained by study day 23 when all treatments were discontinued. This rabbit ate 100% of feed offered daily for the remainder of the study and was euthanized as scheduled. This rabbit has been placed in category E because it can be presumed that this magnitude of body weight loss and decreased feed intake represents more than momentary pain/distress probably as a result of the injection and/or handling on day 1 of the study.

2 rabbits #7884 (vehicle, female) and **#7890** (vehicle, female) had scabs present for a short period of time during the study. #7884 had a scab (3.0 x 1.0 cm) present on the left rear paw on study day 43 prior to injection into the right thigh later on this same day. The scab was no longer present on study day 65. No body weight was lost at any point during the study and feed intake was 100% of the feed offered daily during the entire study. #7890 had a scab (1.5 x 1.5 cm) present on the left rear leg on study day 50. The study day 43 injection was administered to the right leg. No body weight was lost at any point during the study and feed intake was 100% of the feed offered daily during the entire study. These two rabbits have been placed in category E acknowledging that it is possible that more than momentary pain/distress was experienced at some point during the formation of scabs this size on a rabbit. Note: It is unclear if the scabs were related to test article administration, however, we have reported them as if they were, rather than under report Category E.

Study: #14

Animals: 2 Rabbits (high-dose group)

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [ENV/MC/CHEM(98)17]

Diagnosis: 2 rabbits in the highest dosage group (36 mg/kg/day) from this developmental toxicity study conducted in November-December 2005 have been placed in category E as described in the paragraphs below. In the dosage range-finding study conducted at this facility to select doses of this test article to be used in XXXX, scant feces and decreased feed intake occurred in pregnant rabbits dosed at lower dosage levels (12 and 24 mg/kg/day.) The approval states that no adverse signs from test article toxicity were expected. The approval states that no more than momentary pain/distress was expected.

1 rabbit #4469 (high dose; 36 mg/kg/day) has been placed in category E based on the magnitude and duration of weight loss and low to no feed intake prior to euthanasia on the day of abortion. 17% body weight was lost over 15 days and feed intake was low for 13 days. Weight loss of this magnitude and reduced feed intake of this duration can be presumed to be associated with pain/distress. The only other adverse sign present was reduced to no fecal output. The rabbit remained bright, alert and inquisitive. An alfalfa cube was provided daily to encourage improved feed intake. The rabbit was euthanized on the day abortion occurred. The rabbit was normal at necropsy and seven live fetuses and two resorptions were present.

1 rabbit #4465 (high dose; 36 mg/kg/day) from this developmental toxicity study has been placed in category E based on necropsy observations suggestive of accidental dosage administration to the lungs that was likely accompanied by pain/distress at some point prior to death. This rabbit was found dead on gestation day 9 approximately 26 minutes after dosage administration by stomach tube. The rabbit lost 8.8% body weight from gestation day 7 to 9 and had low intake during the same interval. Necropsy revealed red, spongy lung lobes. All other tissues were normal and nine dead embryos were present. Lab management maintains a tracking system of accidental injuries to help identify the need for re-training of any laboratory staff member.

Study: #15

Animals: 1 Rabbit (mid-dose group)

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 1 rabbit #3453 (mid-dosage rabbit) began losing body weight on gestation day 14 (9/22/05) and continued to lose a total 27% body weight through gestation day 25 (10/2/05.) The rabbit's feed consumption during this same time period averaged 27 grams daily (range 64 to 183 grams.) No adverse clinical signs were present during this period. The rabbit was euthanized on gestation day 26 when signs of abortion were apparent. The rabbit was normal on necropsy examination. While no obvious signs of pain or distress were detected prior to abortion and while the rabbit was euthanized at the time of abortion, category E is selected based on the acute nature and the degree of body weight loss.

Study: #16

Animals: 4 Rabbits

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 4 pregnant rabbits from the high dosage group (150 mg/kg/day) have been placed in category E due to body weight loss of a magnitude that can be presumed to be associated with pain/distress. This developmental toxicity study conducted in February-March 2006 was expected to cause more than momentary pain and distress from reduced body weight and reduced feed consumption as a test article effect. The test article was administered by stomach tube. The approval states that animal pain and distress can not be relieved. The approval states that the plan for addressing animals experiencing pain/distress is for the study director and veterinarian to determine at the time of occurrence.

1 rabbit #6075 (150 mg/kg/day) aborted and was euthanized on gestation day 19 (March 5, 2006.) This rabbit lost 18% weight over 11 days and had low feed intake for 8 days that improved to moderate intake for three days prior to the abortion. The only other adverse clinical sign noted for this rabbit was reduced fecal output

1 rabbit #6071 (150 mg/kg/day) aborted and was euthanized on gestation day 22 (March 7, 2006.) This rabbit lost 20% weight over 13 days and had reduced feed intake for 13 days. The only other adverse clinical sign noted prior to abortion was reduced fecal output.

1 rabbit #6076 (150 mg/kg/day) aborted and was euthanized on gestation day 25 (March 12, 2006.) This rabbit lost 24% weight over 16 days and had low feed intake for 12 days that improved to a moderate level of intake for two days prior to the abortion.

1 rabbit #5898 (150 mg/kg/day) was euthanized on gestation day 20 as scheduled for all rabbits assigned to the satellite portion of the study. This rabbit lost 24% weight between gestation days 11 and 16 then maintained weight until euthanasia. Feed intake was reduced for eight days. The only other adverse clinical sign noted for this rabbit was reduced fecal output.

Study: #17

Animals: 1 Rabbit

Type of Study: Oral Embryo-Fetal Developmental Toxicity Studies in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 1 rabbit (#3313; 0 mg/kg/day) from this stomach tube embryo-fetal developmental toxicity study has been placed in category E based on necropsy observations suggestive of an accidental intubation injury that was likely accompanied by pain/distress at some point prior to death. This rabbit was found dead on gestation day 11. No adverse signs were noted prior to death. This rabbit

gained weight daily and was eating well daily.

Necropsy revealed a perforation in one of the right lung lobes and all lung lobes were spongy. All other tissues were normal and eight embryos were present. Lab management maintains a tracking system of accidental injuries to help identify the need for re-training of any laboratory staff member.

Study: #18

Animals: 4 Rabbits (1 in low-dose group and 3 in high-dose group)

Type of Study: Oral Gavage Developmental Toxicity Study of XXX in Female Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 4 rabbits in this developmental toxicity study have been placed in category E as described in the paragraphs below. The approval states that animals in the high dosage groups were expected to experience weight loss and low feed intake. The approval states that animal pain and distress can not be relieved. The approval states that the plan for addressing animals in pain/distress will be decided by the study director and veterinary staff at the time of occurrence.

1 rabbit #4624 (low dosage group) died quickly as a result of dosing error. The rabbit was normal prior to dosage administration by stomach tube. Based on the necropsy findings of mottled lungs and foam in the trachea, it is suspected that some of the test article entered the lungs. Lab management maintains a tracking system for accidental deaths to assist in identifying the need for re-training of any laboratory personnel. While the resulting death was quick, this animal has been placed in category E because the nature of this accidental injury can be presumed to have been associated with pain/distress that some may consider more than momentary.

1 rabbit #4662 (high dosage group) aborted and was euthanized. This rabbit lost 15.8% weight over two weeks and had low feed intake for 12 days that improved to a moderate level of intake the day before the abortion. This rabbit received an alfalfa cube daily beginning six days prior to the abortion to encourage improved feed intake. This rabbit was normal at necropsy and all fetuses were resorptions. This animal has been placed in category E because the magnitude of weight loss and the duration of low feed intake can be presumed to be associated with pain/distress.

1 rabbit #4674 (high dosage group) was found dead on gestation day 19. This rabbit lost 20% weight over 13 days and was consuming little to no feed during this same period. This rabbit received an alfalfa cube daily for six days prior to death to encourage improved feed intake. This rabbit had an ungroomed coat at necropsy and was otherwise normal. All fetuses present were resorptions. This animal has been placed in category E because the magnitude of weight loss and the duration of low feed intake can be presumed to be associated with pain/distress.

1 rabbit #4677 (high dosage group) aborted and was euthanized. This rabbit lost 18% weight over 16 days and was consuming little feed during this time. This rabbit received an alfalfa cube daily for 12 days prior to aborting to encourage improved feed intake. This rabbit was normal at necropsy and the

fetuses present were dead. This animal has been placed in category E because the magnitude of weight loss and the duration of low feed intake can be presumed to be associated with pain/distress.

Study: #19

Animals: 5 Rabbits

Type of Study: Dose Range-Finding Embryo/Fetal Developmental Oral (Gavage) Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 5 rabbits from Part A of this dosage range-finding study have been placed in category E due to body weight loss of a magnitude and low feed intake of a duration that can be presumed to be associated with pain/distress along with adverse clinical signs experienced over the ten day dosage period. This study conducted in June 2006 ended with scheduled euthanasia on the day after the tenth dosage administration. The approval states that animal pain and distress will not be relieved. The approval states that the plan for addressing animals in pain/distress is for the study director and veterinary staff to decide (continuing on study, stoppage of dosage or euthanasia) at the time of occurrence. Veterinary exams were conducted nine times during the ten day dosage period. All rabbits on this study received timothy hay cubes daily to promote improved feed intake.

5 rabbits #7037 (III; 300 MKD), **#7038** (III; 300 MKD), **#7040** (IV; 1000 MKD), **#7041** (IV, 1000 MKD) and **#7042** (IV; 1000 MKD) had low feed intake over the entire ten day dosage period and lost between 18.5% and 20.2% body weight over the same period. All animals remained bright, alert and active. Other adverse signs included scant and/or no fecal output, discolored feces and ungroomed coat. #7041 also had lacrimation from both eyes, ptosis and inflamed eyelids (right eye) on dosage day ten. The eyelids did not appear to be sensitive to touch on veterinary exam. #7042 was euthanized on day 9 of dosage by veterinary recommendation for emaciation and body weight loss of 19.1%. All other rabbits were euthanized as scheduled on the day following the tenth dosage.

Study: #20

Animals: 3 Rabbits

Type of Study: Dose Range-Finding Embryo/Fetal Developmental Oral (Gavage) toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 3 rabbits from Part B of this dosage range-finding study conducted in May-June 2006

have been placed in category E based on the magnitude of body weight loss, the duration of low feed intake and/or behavioral changes. Weight loss of this magnitude and low intake of this duration can be presumed to be associated with pain/distress. The approval states that body weight loss and reduced feed intake are expected at the high dose level (IV; 200 MKD.) The approval states that animal pain and distress can not be relieved. The approval states moribund condition as the endpoint requiring euthanasia. The approval states that additional water sources and timothy hay cubes can be provided to rabbits with weight loss or reduced feed intake. The mid and high dosage groups were evaluated fifteen times by the vet staff for test article effects. And twenty one individual veterinary exams were also completed. All rabbits in group IV (200 MKD) were provided with two water sources and a timothy cube daily from the beginning of dosage administration through the last day of the study.

1 rabbit #4991 (IV; 200 MKD Satellite) had low feed intake and lost 16% body weight from GD7-GD16. Body weight was maintained from GD16-GD20. This rabbit was euthanized as scheduled on GD20. Two water sources and a timothy cube were provided daily from GD8-GD20. Daily grooming was done from GD 12-GD20 to minimize ingestion of loose hair while feed intake was low. This rabbit was examined individually nine times by the vet staff and was also assessed daily as part of the high dosage group from GD8 onward. Pain/distress was noted at exam on GD16 based on the magnitude of weight loss. The rabbit remained bright, alert and inquisitive. The only adverse clinical sign noted for this rabbit was scant fecal output.

1 rabbit #4971 (IV; 200 MKD) lost 14.2% body weight from GD7-GD17 and feed intake was fair to low during this same time. Body weight increased daily from GD17-GD29 and feed intake was good during this same period. Exam on GD 17 noted pain/distress based on magnitude of weight loss and a reduction in inquisitive behavior. Inquisitive behavior returned to normal by GD 19. Reduced fecal output was the only other adverse clinical sign noted. Two water sources and a daily timothy cube were provided from GD8-GD29. This rabbit was euthanized as scheduled on GD29.

1 rabbit #4973 (IV; 200 MKD) lost 11.6% body weight from GD7-GD12, maintained weight from GD 12-GD 15, lost 7% from GD 15-GD 19 and gained daily from GD 19-GD29. Feed intake was low from GD7-GD20 and good from GD20-GD29. Daily grooming was done from GD12-GD21 to minimize ingestion of loose hair while feed intake was low. Pain/distress was noted at exam on GD16 based on the magnitude of weight loss and at exam on GD 18 due to a reduction in inquisitive behavior. Inquisitive behavior returned to normal on GD21. Reduced fecal output was the only other adverse clinical sign noted. Two water sources and a daily timothy cube were provided from GD8-GD29. This rabbit was euthanized as scheduled on GD29.

Study: #21

Animals: 12 Rabbits

Type of Study: Intravenous Dose-Range Finding Embryo-Fetal Development Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 12 rabbits from Part B of this dosage range-finding study (3 Satellite animals and 9 Main study animals) have been placed in category E. All of the rabbits in category E, with one exception,

were from the middle and high dosage groups (100 and 300 mg/kg.) In Part A of this study, there were no test article effects observed in non-mated rabbits that received three intravenous doses of the test article. In Part B of this study, the same dosage levels were selected to be given intravenously five times to pregnant rabbits. Part B was approved with the knowledge that the test article had caused mild to moderate adverse skin reactions in other species. The approval states that animal pain and distress can not be relieved. The approval states the plan for addressing rabbits experiencing pain/distress is for the study director and vet staff to decide on a case-by-case basis at the time of occurrence. All animals on study were evaluated by the veterinary staff ten times during the course of the study for expected test article effects and thirteen individual exams were conducted.

On March 17, 2006, **1 rabbit #6121** (300 mg/kg Main study) was found dead. The day prior to being found dead, the feed intake was reduced. There was no body weight loss and no other adverse signs prior to being found dead. The animal was normal on necropsy exam. It is probable that test article toxicity that caused the death considering the fact that only one of the eight rabbits dosed at the 300 mg/kg level survived until the end of the study. This rabbit has been placed in category E acknowledging that more than momentary pain/distress may have occurred prior to death from test article toxicity.

On March 19, 2006, **2 rabbits #6119** (100 mg/kg Main study) and **#6125** (300 mg/kg Satellite) were found dead. #6119 lost 5% body weight over five days and had reduced feed intake for four days. At necropsy, the kidneys were pale. #6125 lost 6% body weight over four days and had low feed intake for four days. This rabbit was normal at necropsy except for an ungroomed coat. It is probable that test article toxicity caused these deaths considering the high mortality rate at the 100 mg/kg and 300 mg/kg levels. These rabbits have been placed in category E acknowledging that more than momentary pain/distress may have occurred prior to death from test article toxicity.

On March 20, 2006, **2 rabbits #6124** (300 mg/kg Main study) and **#2591** (300 mg/kg Main study) were found dead. #6124 lost 5% body weight over four days and had reduced feed intake for four days. Necropsy examination showed an eroded and ulcerated stomach lining and mottled lungs. #2591 lost 7% body weight over four days and had reduced feed intake for four days. Necropsy exam showed mottled lungs. These rabbits have been placed in category E due to the likelihood that pain/distress was associated with the drug toxicity that caused their deaths and with the necropsy findings. Note: On March 20, 2006, all remaining animals in the 300 mg/kg groups were provided with an alfalfa cube to encourage improved feed intake.

On March 22, 2006, **4 rabbits** were found dead, **#6118** (100 mg/kg Main study), **#6123** (300 mg/kg Main study) and **#6890** (300 mg/kg Satellite) and one animal, **#6888** (100 mg/kg Satellite) was euthanized on the date the satellite portion of the study was scheduled to end. These rabbits have been placed in category E due to the magnitude of acute body weight loss and/or behavioral changes that can be presumed to be associated with pain/distress. #6118 was examined on March 21 for 17% body weight loss over seven days, five days of reduced feed intake. The rabbit was also not consuming the alfalfa cubes being provided. Other clinical signs included labored breathing and perinasal substance. #6123 was examined on March 21 for 14% body weight loss over seven days, reduced alertness and responsiveness and four days of reduced feed. Other signs included labored breathing, head tilt and scant feces. #6890 lost 19% body weight over six days and had six days of reduced feed intake. #6888 lost 16% body weight over seven days and had seven days of low feed intake. This rabbit was examined on March 20 for 4% body weight loss and reduced alertness and alfalfa cubes were provided. On March 21, the rabbit was normal in behavior and attitude and had lost an additional 4% body weight. On exam on March 22, behavior changes indicated pain and distress and the rabbit was euthanized as scheduled according to the protocol on that date.

On March 23, 2006, **2 rabbits #6116** (100 mg/kg Main study) and **#6120** (100 mg/kg Main study) were found dead. #6116 lost 27% body weight over 12 days and had eight days of reduced feed intake. This rabbit has been placed in category E due to the magnitude of acute body weight loss and duration of the low feed intake that can be presumed to be associated with pain/distress. #6120 lost 5% body weight over five days and had reduced feed intake for four days. Necropsy exam showed pale kidneys. This rabbit has been placed in category E due to the likelihood that pain/distress was associated with the drug toxicity that caused death and with the necropsy findings.

On March 28, 2006, **1 rabbit #6111** (30 mg/kg Main study) was euthanized due to adverse signs that included 21% body weight loss over eight days, eight days of reduced feed intake, dehydration, decreased responsiveness and scant feces. This rabbit has been placed in category E due to the magnitude of acute body weight loss and duration of the low feed intake that can be presumed to be associated with pain/distress.

Study: #22

Animals: 14 Rabbits

Type of Study: Oral (Stomach Tube) Dose-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 7 non-mated rabbits from Part A of this dosage range-finding study have been placed in category E based on the nature and duration of adverse clinical signs and/or the extent and duration of reduced feed intake and body weight loss as described in the following paragraphs. In this study conducted in March 2006, the test article was administered once daily for ten days by stomach tube. This test article had not been given to rabbits before. Rats given dosages above 100 mg/kg experienced lethargy, ungroomed coats, GI tract lesions and morbidity. The dosages selected were 0, 10, 100, 300 and 500 mg/kg/day. There were three rabbits assigned to each dosage group. Eleven veterinary exams were performed during the ten day dosage period. The study was approved with knowledge of the potential for more than momentary pain/distress and stated that animal pain/distress can not be relieved. The approved plan for addressing any animal experiencing pain/distress was for the study director, sponsor and veterinarian to discuss and decide at the time of occurrence.

3 rabbits # 6993 (III, 100 MKD), **#6994** (III, 100 MKD) and **#6995** (IV, 300 MKD), lost 15.6%, 14.6% and 19% body weight respectively over the ten day dosage period. The feed intake was also low during this same period. Clinical signs for #6993 and #6994 included soft and/or scant feces and ungroomed coat. Clinical signs for #6995 included scant feces, ungroomed coat, splayed limbs and head tilt. These rabbits were euthanized according to the study schedule on the day after the last dosage administration.

1 rabbit #6996 (IV, 300 MKD) lost 19% body weight in the first 8 days of the dosage period prior to euthanasia by veterinary recommendation on day 8 of the study. Feed intake was low during this same period. This animal was examined three times prior to euthanasia and was provided with a timothy cube daily to promote improved feed intake. The day prior to euthanasia, the rabbit was alert with

ataxia and light pink mucus membranes. On the day of euthanasia, clinical signs included head tilt, cold to touch, pale, lacrimation, splayed limbs, mydriasis and decreased motor activity.

2 rabbits #6998 (V, 500 MKD) and **#7000** (V, 500 MKD) lost 17.6% and 16.5% body weight respectively over the first five days of the dosage period prior to euthanasia by veterinary recommendation on day 5 of the study. Feed intake was low to none during this same period. These rabbits were examined several times prior to euthanasia and were provided with timothy cubes daily to promote improved feed intake. These rabbits were also groomed daily to minimize loose hair that could be consumed during grooming. Adverse signs for these rabbits beginning at least one day prior to euthanasia included scant feces, ungroomed coat, head tilt, rapid respiration, splayed limbs and decreased motor activity. On the day of euthanasia, adverse signs included lacrimation, limited use of limbs, head tilt, ataxia, decreased motor activity, cold to touch, excess salivation, muscle rigidity and lost righting reflex.

1 rabbit #6999 (V, 500 MKD) lost 13% body weight over the first seven days of the study prior to euthanasia by veterinary recommendation on day 7 of the study. Feed intake was moderate on day 1 of the study and low to none from day 2 until euthanasia. This rabbit was examined several times prior to euthanasia and was provided with a timothy cube daily to promote improved feed intake. This rabbit was also groomed daily to reduce loose hair that could be consumed during grooming. Adverse signs prior to the day of euthanasia were scant feces and ungroomed coat. Veterinary exams including one on the day before euthanasia indicate this rabbit was alert, active and inquisitive. At exam on the morning of euthanasia, adverse signs included excess salivation, cold to touch, limited use of limbs, lacrimation, perinasal substance and decreased motor activity. Even though this animal was euthanized on the same date that adverse signs were first observed, this animal was placed in category E because there is no way of knowing the duration of the clinical signs in the overnight period before euthanasia that morning.

7 pregnant rabbits in Part B of this dosage-range finding study conducted in April 2006 have been placed in category E based on the nature and duration of adverse clinical signs and/or the extent and duration of reduced feed intake and body weight loss as described in the following paragraphs. The dosage levels selected, 0, 3, 10, 30, and 100 mg/kg/day, were based on the results of Part A of this study. (In Part A, two of three rabbits at 100 mg/kg/day were placed in category E for moderate to severe acute body weight loss and adverse neurological signs.) The test article was administered daily by stomach tube from day 7 through day 19 of gestation. Twenty seven veterinary exams were performed during the conduct of this study. The study was approved with knowledge of the potential for more than momentary pain/distress associated with expected body weight loss at the higher dosage levels. The approval stated that animal pain and distress can not be relieved. Also approved was a plan for addressing any animal experiencing pain/distress which was for the study director, sponsor and veterinarian to discuss and decide at the time of occurrence.

1 rabbit #6506 (group II, 3 MKD) was found dead during the morning viability check. No adverse clinical signs and no weight loss or reduction in feed intake occurred prior to being found dead. Necropsy examination revealed a perforation in one lung lobe, mottled discoloration of all lung lobes and red perinasal substance. This perforation was likely the result of an accidental injury during dosage administration by stomach tube the day before death. The lab technician involved is experienced in this procedure and had no history of problems with this procedure in rabbits. Incidents of this nature are tracked by laboratory management in order to identify patterns which may indicate the need for re-training of any particular member of the laboratory staff.

1 rabbit #6532 (group IV, 30 MKD) lost 19% body weight from gestation day 8 to 16 and was euthanized by veterinary recommendation on gestation day 17. This animal's feed intake was low during this same period. All rabbits in groups IV and V were provided with a timothy cube daily beginning on

gestation day 14 to promote improved feed intake. This rabbit was examined several times prior to euthanasia. Beginning on gestation day 14, this rabbit was noted as alert and responsive but not inquisitive possibly indicating pain/distress. Scant fecal output and ungroomed coat were also noted. This rabbit was normal on necropsy exam. All fetuses were resorptions.

2 rabbits #6535 (group V, 100 MKD) and **#6536** (group V, 100 MKD) lost 16.4 and 16.5 % body weight, respectively, from gestation day 7 to 20 prior to scheduled euthanasia on gestation day 20. Feed intake was fair on gestation day 7 to 8 then low to none from gestation day 8 to 20. All rabbits in groups IV and V were provided with a timothy cube daily beginning on gestation day 14 to promote improved feed intake. While monitoring the weight loss in this study, rabbits in groups IV and V were examined several times. Adverse signs noted from gestation day 11 until euthanasia were scant/soft feces and ungroomed coat. Rabbit #6535 aborted on the same day as euthanasia was scheduled to occur according to the protocol.

1 rabbit #6525 (group V, 100 MKD) lost 16.8 % body weight from gestation day 7 to 21 prior to abortion and euthanasia on gestation day 22. Feed intake was low from gestation day 8 to 20 then fair from gestation day 20 to 21. All rabbits in groups IV and V were provided with a timothy cube daily beginning on gestation day 14 to promote improved feed intake. While monitoring the weight loss in this study, rabbits in groups IV and V were examined several times. Adverse signs noted from gestation day 11 until euthanasia were scant/soft feces and ungroomed coat.

2 rabbits #6523 (group V, 100 MKD) and **#6524** (group V, 100 MKD) lost 19.8 and 19.0 % body daily, respectively, from gestation day 8 through day 21 (#6523) and through day 23 (#6524.) Both rabbits aborted and were euthanized on the day of abortion. Feed intake was low during this same period and improved to fair in #6524 from gestation day 22 to 23. All rabbits in groups IV and V were provided with a timothy cube daily beginning on gestation day 14 to promote improved feed intake. #6523 was examined daily from gestation day 16 to 22. Apprehensive behavior possibly indicating pain/distress was noted on gestation days 16 and 20 while remaining alert and responsive. Other adverse signs included scant feces and ungroomed coat. Dehydration was also noted on the day of abortion. #6524 was examined several times prior to abortion. Apprehension possibly indicating pain/distress was noted on several of these exams. Dehydration was also noted and as a result, the timothy cube already being provided daily was then moistened with water and provided in a bowl. Other adverse signs included scant feces and ungroomed coat.

Study: #23

Animals: 10 Rabbits

Type of Study: Oral (Stomach Tube) Dose-Range Developmental Toxicity Study of XXX in New Zealand White Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 10 rabbits from this dosage range-finding developmental toxicity study conducted in May-June 2006 have been placed in category E for the reasons described in the paragraphs below. The dosage levels administered from gestation 7 through 19 were 0, 100, 200, 350 and 500 mg/kg/day. The

only expected adverse clinical sign was red ears. This study was approved with the knowledge that six dosages of this test article at 200 mg/kg/day significantly increased the hematocrit and hemoglobin levels in rabbits. The approval states that animal pain/distress can not be relieved and that the approved plan for addressing rabbits experiencing pain/distress was for the study director, sponsor and veterinarian to determine at the time of occurrence.

2 rabbits #7419 (350 MKD) and **#7422** (500 MKD) were found dead on GD8 during the morning room check on the day following the first dosage administration on GD7. Weight loss from GD7 to death was 5-6%. One rabbit was normal on necropsy exam and the other had several white areas on the heart and subcutaneous blood in the area of the ventral thorax.

1 rabbit #7420 (350 MKD) was examined and determined to be in moribund condition on GD8 during the morning room check the day following the first dosage administration on GD7. Adverse signs included cold to touch, ataxia, lethargy, splayed limbs, tachypnea and hyperreactivity. This animal lost 2.4% body weight in 24 hours. This rabbit was euthanized. The spleen was noted as small at necropsy. These three rabbits have been placed in category E because it is possible that adverse signs associated with pain/distress existed for some period of time in the overnight hours prior to death or prior to onset of moribund condition.

3 rabbits #7418 (350 MKD), **#7421** (500 MKD) and **#7440** (500 MKD) were found dead on GD8 approximately two hours following the second dosage administration. Clinical signs included ataxia, perinasal substance, tachypnea, and decreased motor activity. Weight loss was 3.5-4.4% and feed intake was reduced to a low/fair level over the previous 24 hour period. Necropsy observations included subcutaneous blood in the areas of the ventral thorax and ventral abdomen, blood in areas around the brain, blood in the pericardial sac, red areas on the thymus, white areas on the heart and red uteri. These rabbits have been placed in category E because it can be presumed pain/distress was associated with the adverse clinical signs and the adverse necropsy signs prior to the occurrence of death.

1 rabbit #7414 (200 MKD) was euthanized on GD11 per veterinary recommendation due to severe body weight loss and adverse clinical signs. Adverse clinical signs included ptosis, lacrimation, hyperpnea, scant fecal output and cold to touch. Beginning on GD8, this animal was examined daily and provided with two water sources and a timothy cube daily to help improve feed intake. Weight loss from GD7 to GD 10 was 16% and from GD7 to euthanasia on GD11 was 26%. Feed intake was low from GD7 to euthanasia. Necropsy findings included blood in areas around the brain, red areas on the thymus, a pale kidney and white areas on the heart. All fetuses were resorptions. This rabbit has been placed in category E because it can be presumed pain/distress was associated with the adverse clinical signs including weight loss and the adverse necropsy signs prior to euthanasia.

1 rabbit #7412 (200 MKD) were found dead on GD12 during the morning room check. Body weight loss was 8.9% from GD7 to GD 11 and feed intake was low during this same period. Beginning on GD8, this animal was examined daily and provided with two water sources and a timothy cube daily to help improve feed intake. Adverse signs present one to two days prior to death were soft feces, ungroomed coat and discolored urine. The necropsy exam revealed white areas on the heart. This rabbit has been placed in category E because it is possible that adverse signs associated with pain/distress existed for more than a momentary period of time in the overnight hours prior to death.

1 rabbit #7430 (100 MKD) aborted and was euthanized on GD20. This rabbit lost 20% body weight from GD9 to GD 19. Feed intake was good from GD7 to GD 13 then low from GD13 to euthanasia. This animal was examined daily and provided with two water sources and a timothy cube daily to help improve feed intake. Adverse clinical signs were reduced fecal output and discolored urine. The

rabbit remained bright, alert, responsive and inquisitive until exam on the day of abortion when timid behavior possibly indicating pain/distress was noted. The necropsy exam revealed a dark red spleen, blood in areas around the brain and all fetuses were resorptions. This rabbit has been placed in category E because it can be presumed pain/distress was associated with the adverse clinical signs including weight loss and the adverse necropsy signs prior to euthanasia.

1 rabbit #7429 (100 MKD) had several periods of body weight gain interspersed with several periods of weight loss from GD7 to GD21. The gains and losses approximately equaled one another until a period of weight loss began on GD18 resulting in 17.4% weight loss by GD21. Feed intake was good from GD7 to GD10 then low from GD10 to GD21. This animal was examined daily and provided with two water sources and a timothy cube daily to help improve feed intake. Adverse clinical signs were reduced fecal output and ungroomed coat. This rabbit remained bright, alert, responsive and inquisitive and was euthanized on the day of scheduled euthanasia. This rabbit has been placed in category E because it can be presumed pain/distress was associated with body weight loss of the magnitude experienced prior to euthanasia.

Study: #24

Animals: 16 Rabbits

Type of Study: Oral (Stomach Tube) Dose-Range Developmental Toxicity Study of XXX in New Zealand White Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 16 rabbits from this dosage range-finding developmental toxicity study conducted in June-July 2006 have been placed in category E for the reasons described in the paragraphs below. The test article was given by stomach tube on gestation days 7, 9, 11, 13, 15, 17, and 19. There were eight rabbits assigned to each of five dosage levels, 0, 150, 200, 300 and 400 mg/kg/day. More than 58 veterinary exams were completed during the conduct of this study. This study was approved with the knowledge that the test article given at 200 MKD significantly increased both hematocrit and hemoglobin levels. In a previous dosage-range finding developmental toxicity study at this facility using this same test article given to rabbits on gestation days 7 through 19, one dose at 500 MKD resulted in the death or euthanasia of all rabbits within approx. 8-28 hours, one to two doses at 350 MKD resulted in the death or euthanasia of all rabbits within approx. 24-33 hours and five to six doses at 200 MKD resulted in the euthanasia of all rabbits by the seventh daily dosage. Approved measures for animals experiencing pain/distress were additional water sources, timothy cubes or possible euthanasia to be decided by the study director and vet staff at time of any occurrence.

June 21, 2006. **1 rabbit #7933** (III; 200 MKD) died on GD7 during blood collection approximately twelve hours after the first dosage administration. This animal appeared normal approximately four hours prior to death during an exam to check the entire study for adverse effects. Seizures, periorbital substance and loss of righting reflex were noted prior to death. Necropsy exam showed areas of hemorrhage around the brain, lungs mottled red to dark red, thymus with red areas and a dark red trachea. These findings were the same as those seen in the rabbits that died or were euthanized in the previous study using the same test article at this dosage level. This rabbit has been placed in category

E because it can be presumed that pain/distress was associated with the processes leading to the adverse necropsy signs prior to the occurrence of death.

June 22, 2006. **1 rabbit #7938** (V; 400 MKD) was found dead on GD8 during the morning room check approximately 22 hours after the first dosage administration. No adverse clinical signs were noted prior to death and study staff were present until approximately 9 p.m. the night before. No body weight or feed intake data was recorded. The necropsy exam showed subcutaneous hemorrhage in the area of the ventral abdomen, the thymus had many red areas and there were white areas present on the heart. These findings were the same as those seen in the rabbits that died or were euthanized in the previous study using the same test article at similar dosage levels (200, 350 and 500 MKD.) This rabbit has been placed in category E because it is not possible to determine whether or not adverse clinical signs indicative of pain/distress occurred during the overnight hours prior to death and because it can be presumed that pain/distress was associated with the processes leading to the adverse necropsy signs prior to death.

June 22, 2006. During the morning veterinary exam approximately 22 hours after the first dosage administration, the majority of rabbits in groups III-V were alert but showed no interest in surroundings or in approach for handling. By veterinary recommendation, two water sources and timothy cubes were provided to all rabbits in groups III-V. Because similar adverse clinical signs and necropsy findings were seen in the previous study and because the test article was known to increase hematocrit levels, it was recommended that in-house hematocrit levels be done on rabbits in groups III-V in order to aid in determining an endpoint before death or moribund condition occurred. During the afternoon exam, many of the rabbits in groups III-V were alert but continued to show no interest in surroundings or in approach for handling and their feed intake was moderately to severely reduced.

3 rabbits #7934 (III; 200 MKD), **#7923** (V; 400 MKD) and **#7939** (V; 400 MKD) were euthanized in the early evening by veterinary recommendation. Lack of interest in surroundings or in handling was present since the morning and scant fecal output was also noted. Weight loss ranged from 2.0-3.1% over 24 hours. Necropsy findings included pale kidneys, subcutaneous hemorrhage in the area of the ventral chest and ventral abdomen, lungs spongy, lungs mottled red to dark red, red areas on the thymus, thick liver lobes, blood around the mouth and nose, hemorrhage around the brain and stomach erosions. These rabbits have been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs present for at least 12 hours and with the processes leading to the adverse necropsy signs prior to euthanasia.

June 23, 2006. After one dosage on June 21, 2006, only four rabbits of eight remained alive in the 400 MKD group by the time of the morning veterinary exam on GD9. **3 rabbits #7921** (V; 400 MKD), **#7922** (V; 400 MKD) and **#7940** (V; 400 MKD) were uninterested in surroundings, reluctant to move, tense on examination and tended to focus on respiration. Euthanasia was recommended. Approximately four hours after the exam, the rabbits were euthanized. The remaining rabbit at this dosage level, **1 rabbit #7924** (V; 400 MKD), was also euthanized. Weight loss from GD7 to GD9 ranged from 10.0 to 13.6 %. Necropsy findings included pale kidneys, mottled lungs, hemorrhage around the brain, mesenteric lymph nodes large and red, thick liver lobes, stomach erosions, subcutaneous hemorrhage in the area of the ventral chest and abdomen, black spleen and pink foam in the trachea. These four rabbits have been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs present for at least four hours and with the processes leading to the adverse necropsy signs prior to euthanasia.

June 25, 2006. **1 rabbit #7935** (IV; 300 MKD) was found dead on GD11 during the morning room check. This rabbit had received two doses of the test article. 9.1% body weight was lost since GD7. Feed intake was moderate from GD7 to GD9 and low from GD9 to GD 10. Two water sources and a daily timothy cube were provided beginning on GD8. Timid behavior was noted during two exams on

GD8 and subcutaneous fluids were administered on GD8. Behavior was normal during exams on GD9 and GD10. Necropsy exam revealed pale kidneys and all lung lobes were red. This rabbit has been placed in category E because it is not possible to determine whether or not adverse clinical signs indicative of pain/distress occurred in overnight hours prior to death and because it can be presumed that pain/distress was associated with the processes leading to the adverse necropsy signs prior to death.

June 25, 2006. **1 rabbit #7936** (IV; 300 MKD) was euthanized by veterinary recommendation on GD11. This rabbit had received two doses of test article. At the time of the morning veterinary exam, slow respiration and decreased activity level were noted. Reduced interest in surroundings and reduced activity were also noted on GD8 and GD9. Behavior was normal during exams on GD 10. 17% body weight was lost from GD8 to GD 11 and feed intake was low from GD7 to GD 11. Two water sources and a timothy cube daily were provided beginning on GD8. Necropsy revealed pale kidneys and stomach erosions. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs and with the processes leading to the adverse necropsy signs prior to euthanasia.

June 26, 2006. **1 rabbit #7937** (IV; 300 MKD) was found dead on GD12 during the morning room check. This rabbit had received three doses of the test article. 16% body weight was lost from GD7 to GD 11 and feed intake was low during this same period. Other adverse signs included scant feces on GD10-GD11 and slow respiration was noted on GD10. Two water sources and a daily timothy cube were provided beginning on GD8. All tissues appeared normal at necropsy examination. This rabbit has been placed in category E because the test article given at 300 MKD and higher had a history of causing morbidity/mortality in rabbits and consequently it is reasonable to presume that adverse clinical signs indicative of pain/distress occurred in the overnight hours prior to death.

June 27, 2006. **1 rabbit #7919** (IV; 300 MKD) was euthanized by veterinary recommendation on GD13. This rabbit had received three doses of the test article when slow respiration and decreased activity were noted during the morning veterinary exam. This rabbit was examined daily and respiration was noted as either slow or with increased effort since GD10. A second water source and a daily timothy cube were provided beginning on GD8. Subcutaneous fluids were administered and a water-soaked timothy cube was provided on GD 12. Weight loss from GD7 to GD13 was 23.9% and feed intake was low from GD7 to GD13. At necropsy, all fetuses were resorbed, the mesentery was pink, mesenteric lymph nodes were red and Peyer's patches were red. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs including weight loss of the magnitude experienced and with the processes leading to the adverse necropsy signs prior to time of euthanasia.

June 27, 2006. **1 rabbit #7916** (IV; 300 MKD) was euthanized by veterinary recommendation on GD13. This rabbit had received three doses of test article when a reduced interest in surroundings was noted during the morning exam. 16% weight loss from GD7 to GD13 was also noted. Daily grooming and an afternoon follow-up exam were recommended. A second water source and daily timothy cube were provided beginning on GD8. Ptosis, aggression and vocalization were noted during the afternoon exam so the rabbit was euthanized. At exam on the day before euthanasia, pain/distress was noted due to vocalization during the exam and due to 14% weight loss over a five day period. Necropsy exam revealed stomach erosions and all fetuses were resorbed. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs including weight loss of the magnitude experienced and with the processes leading to the adverse necropsy signs prior to time of euthanasia.

June 28, 2006. **1 rabbit #7929** (II; 150 MKD) was euthanized by veterinary recommendation on GD15.

This rabbit had received four doses of the test article. During acclimation, a second water source, a daily timothy cube and daily grooming were initiated because feed intake was greatly reduced on GD3 to GD4 and was zero from GD4 to GDS. On GDS, vet staff recommended replacement of this rabbit with the available unassigned rabbit due to the low feed intake. On GD6, feed intake was improved. Dosage administration began on GD7. Seven additional exams were done from GD9 to GD15. The rabbit remained bright, alert and active through the morning exam conducted on GD15. Later that same morning, a change in activity level was noted along with discomfort on abdominal palpation. Ptosis and cold extremities soon followed and euthanasia was performed. Periods of weight loss and periods of weight gain occurred during the dosing period for an overall loss of 15% from GD7 to GD15. Feed intake levels also fluctuated during this period. Necropsy exam revealed pericardial fluid, a black spleen, an edematous thymus, green fluid in the duodenum and dark red cecal mucosa. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs including weight loss and with the processes leading to the adverse necropsy signs prior to time of euthanasia.

July 3, 2006. **1 rabbit #7932** (III; 200 MKD) was euthanized on GD19 in the afternoon following dosage administration. This rabbit had received seven doses of the test article. A second water source and a daily timothy cube were provided beginning on GD8. Slow respiration and decreased activity level were noted during the morning veterinary exam prior to dosage on GD19. By the afternoon, there was no improvement so the rabbit was euthanized. 20.8% body weight was lost from GD11 to GD19. Feed intake was fair from GD 12 to GD 13 and low from GD 13 to GD18. On GD 17, the rabbit was noted as bright, alert and responsive on exam with 15% weight loss. A water-moistened timothy cube was provided. At the GD18 exam, the rabbit remained bright, alert and responsive and there was no additional weight loss. Feed intake remained low and three other rabbits at this dosage level had already died or been euthanized so subcutaneous fluids were administered. Necropsy exam revealed a black spleen, stomach ulcerations, red lymph nodes and Peyer's patches and all fetuses were resorbed. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the adverse clinical signs including weight loss and with the processes leading to the adverse necropsy signs prior to the time of euthanasia.

Study: #25

Animals: 4 Rabbits

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 4 rabbits from this developmental toxicity study conducted in February 2006 have been placed in category E due to the nature of or the duration and/or magnitude of the clinical signs including body weight loss and low feed intake. The study was approved with the knowledge that the test article might cause more than momentary pain/distress as a result of reduced feed intake and body weight loss. The approved plan for addressing rabbits experiencing pain/distress was for the study director and vet staff to decide at the time of occurrence. Rabbits were dosed for 13 days (gestation day 6 through 18.) Twelve exams were completed during the conduct of this study.

1 rabbit #5519 (group I- Main study) died 11 minutes after attempted dosage administration. The test article was not administered. At necropsy, a torn lung lobe was noted. The rabbit was normal prior to this event. The accident was a first time occurrence for the technician involved. Lab management maintains a tracking system for monitoring accidental events in order to help identify the need for re-training of any laboratory staff member. Going forward, this technician was placed on a monitoring list for any additional indications of dosage problems.

1 rabbit #5586 (group 4-high dose- Main study) aborted and was euthanized on gestation day 20. Prior to the abortion, this rabbit lost 20% weight over the 13 day dosage period and had low feed intake over the same time period. Rabbit was examined the day prior to aborting at the request of study staff for weight loss and was otherwise normal in behavior and attitude. A second water source was provided by staff prior to the exam. At exam, the weight loss was incorrectly calculated to be 10%. A correct calculation of 20% would have resulted in the recommendation of euthanasia based on the magnitude of weight loss at the time of exam. An alfalfa cube was provided at exam as an effort to improve feed consumption now that the dosage period had ended and re-exam was scheduled for GD20. Tan areas were present on two lung lobes at necropsy exam and six live fetuses and two early resorptions were present.

1 rabbit #5623 (high dose- Satellite) aborted and was euthanized on gestation day 19 as scheduled for the satellite portion of this study. Prior to abortion, the rabbit lost 17% weight over twelve days and had reduced feed intake over the same period. Scant fecal output was noted on gestation days 17-19. The rabbit had no other adverse clinical signs. The rabbit was normal at necropsy exam.

1 rabbit #2875 (high dose- Satellite) was euthanized on gestation day 19 as scheduled for the satellite portion of this study. Prior to euthanasia, the rabbit lost 18% weight over the thirteen day dosage period and had low feed intake for 10 of those thirteen days. Scant fecal output was noted on gestation days 17-19. The rabbit had no other adverse signs. Only pregnancy status was recorded at scheduled sacrifice. As per the protocol, no examination was performed at scheduled euthanasia for rabbits assigned to the satellite portion of the study.

Study: #26

Animals: 2 Rabbits

Type of Study: Subcutaneous Dosage Range Development Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 2 rabbits from Part A of this subcutaneous dosage range-finding developmental toxicity study conducted in May 2006 have been placed in category E as described for each rabbit in the paragraphs below. The test article was given once on days 1, 3, 5, 7, 9, 11 and 13 of the study. There were seven injection sites used on a rotating basis so no site was used more than once. Euthanasia was scheduled for day 14 of the study. The skin was monitored by Draize scoring daily before dosage and again one hour after dosage (on the days of dosage) and nonce daily on the days when no test article was administered. This study was approved with the knowledge that local swelling and ulceration could

result from the test article at the highest dosage level (50 mg/kg). The approved endpoint was moribund condition.

1 rabbit #6555 (group II; mg/kg) was examined on day 8 of the study for an ulceration measuring 2.5 cm in diameter at injection site B. Site A and D also had ulcerations measuring 1.0 cm in diameter and 2.0 x 1.5 cm, respectively. Manipulation of the sites elicited no adverse reaction, behavior was normal, feed intake was 100% daily and there was no body weight loss. At re-exam prior to dosage the next day (day of study 9), the rabbit appeared apprehensive but was not reactive to manipulation of the sites, ate 100% of feed overnight and gained weight. A cold compress was applied for approximately 10-15 minutes immediately after dosing at the sites dosed on days 9, 11 and 13 of the study. Re-exams were conducted daily on this rabbit until the end of study. No adverse reaction to manipulation of the dosage sites was present, feed intake remained at 100% daily and there were daily body weight gains. This rabbit was placed in category E based on the apprehensive behavior noted prior to dosage on day 9 of the study and because the inflammatory process necessary to produce ulcerations of the size noted can be presumed to be associated with pain/distress even when not evident by manipulation.

1 rabbit #6561 (group IV; 50 mg/kg). The injection site used on day 1 of the study had a scab present on day 3 through day 12 of the study (no size was recorded in the data) and two other injection sites were red/purple for a two to four day period. Feed intake was good on all days of study on which the rabbit was not injected. On the days of injection, feed intake was reduced by 10-80%. The rabbit gained body weight on the days of the study on which the rabbit was not injected and had minor body weight losses (4% or less) on days of the study on which injections occurred. This rabbit was placed in category E because of the pattern of feed intake and weight loss suggests a cycle of pain/distress in direct correlation with the administration of the test article at a dosage level that resulted in early euthanasia of all other rabbits in the group.

Study: #27

Animals: 9 Rabbits

Type of Study: Subcutaneous Dosage-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: **9 rabbits** from Part B of this dosage range-finding developmental toxicity study conducted in June 2006 have been placed in category E as described for each rabbit in the paragraphs below. The test article was injected subcutaneously on gestation days 7, 9, 11, 13, 15, 17 and 19 (gestation days 7, 10, 13, 16 and 19 for three animals assigned to TK in group V) at 0 (sham), 0 (vehicle), 1, 5 and 10 mg/kg. There were seven injection sites used on a sequential basis so no site was used more than once. The skin was Draize scored daily before dosage and again one hour after dosage (on the days of dosage administration) and once daily on the days the test article was not administered. More than thirty veterinary exams were completed. Part B was approved with the knowledge that local swelling and ulceration from every other day administration of the test article occurred at 5 mg/kg and resulted in the euthanasia of all rabbits at the 10 mg/kg dosage level prior to the scheduled end date of Part A. Approved endpoint for rabbits with multiple necrotic lesions or ulcerations, infected ulceration,

vocalization or depression was a dosing holiday or euthanasia to be decided by study director and vet staff at time of occurrence.

1 rabbit #7812 (1 mg/kg) had an ulceration at two injection sites. One measured 2.0 x 1.0 cm and the other 1.0 x 0.5 cm. The rabbit gained body weight daily and had good daily feed consumption levels. This rabbit has been placed in category E because the inflammatory process necessary to produce ulcerations of the sizes noted may be presumed to have been associated with pain/distress at some point during the formation..

1 rabbit #7813 (1 mg/kg) had a scab present on the right ear (1.5 x 0.3 cm) at arrival that persisted until healed more than two weeks later. There was no pain/distress noted at the exam conducted at arrival or at the two re-check exams. The rabbit gained body weight daily and had good daily feed consumption levels. This rabbit has been placed in category E because the exams performed acknowledge that the inflammatory process necessary to produce a scab of the size noted may be presumed to have been associated with pain/distress at some point during formation.

1 rabbit #7816 (5 mg/kg) had an ulceration (1.0 x 1.0 cm) present over the left hip at veterinary exam. Based on the fact the ulceration was in an area where the skin moves during locomotion, the exam form acknowledges that pain/distress may have been experienced at some point during the inflammatory process necessary to produce the ulceration. This rabbit had good daily feed consumption levels and gained weight daily.

1 rabbit #7820 (5 mg/kg) had ulcerations at four injections sites ranging in size from 0.5 cm in diameter to 3.0 x 0.4 cm. There was no pain/distress noted at the initial exam or during the re-check exams. The areas were clipped and cleaned and healed well. This rabbit had good daily feed consumption levels and gained body weight daily. This rabbit has been placed in category E because the exams acknowledge that the inflammatory process necessary to produce ulcerations of the sizes noted may be presumed to have been associated with pain/distress at some point during formation.

1 rabbit #7822 (10 mg/kg) was examined for an abrasion 1.5 x 1.5 cm at one injection site on gestation day 12. The rabbit vocalized during exam. The site was clipped and cleaned and coated with KY® Jelly. At re-exam the next day and again in two days, the rabbit appeared timid but was non-reactive when the area was touched. The abrasion healed well. The rabbit gained or maintained body weight daily. Feed intake was slightly reduced GD13-16. This rabbit has been placed in category E because the exams acknowledge that the timid behavior may be indicative of pain/distress and the inflammatory process necessary to produce an abrasion of the size noted may be presumed to have been associated with pain/distress.

1 rabbit #7823 (10 mg/kg) was examined for an abrasion 4.0 x 3.0 cm on the right side of the body. The site was clipped and cleaned and coated with KY® Jelly at that exam and again for the next three days. At one re-check exam, an ulceration (2.0 cm in diameter) was noted adjacent to the abrasion. This increased to 3.0 cm in diameter over the next few days and then healed well. This rabbit gained or maintained body weight daily and had good daily feed consumption levels. This rabbit has been placed in category E because the exams acknowledge that the inflammatory process necessary to produce the abrasion and the ulceration of the sizes noted may be presumed to have been associated with pain/distress before treatment was initiated.

1 rabbit #7826 (1 mg/kg) was examined for an ulceration 4.0 x 1.5 cm at one injection site on gestation day 20. The rabbit vocalized and attempted to bite during the exam. The rabbit ate 100% every day of the study and gained body weight daily. The rabbit was euthanized as scheduled the following day on GD21. This rabbit has been placed in category E because the exam acknowledges that the aggressive

behavior may be indicative of pain/distress and the inflammatory process necessary to produce an ulceration of the size noted may be presumed to have been associated with pain/distress.

1 rabbit #7830 (5 mg/kg) was examined for an ulceration 1.5 x 1.5 cm at one injection site. No pain/distress was noted at exam or at any of the subsequent three re-exams. The rabbit ate 100% every day of the study and gained weight daily. This rabbit has been placed in category E because the exam acknowledges that the inflammatory process necessary to produce an ulceration of the size noted may be presumed to have been associated with pain/distress.

1 rabbit #7837 (10 mg/kg on GD 7, 10, 13, 16 and 19) had an ulceration at one injection site measuring 4.0 x 1.0 cm beginning on GD15. This rabbit gained weight daily and had good to slightly reduced feed intake during the study. This rabbit was euthanized as scheduled on GD21. This rabbit has been placed in category E because the inflammatory process necessary to produce an ulceration of the size noted may be presumed to have been associated with pain/distress.

Study: #28

Animals: 3 Rabbits

Type of Study: Oral (Stomach Tube) Dosage-Range developmental Toxicity Study of XXX in Rabbits
Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 3 rabbits from this dosage range-finding developmental toxicity study conducted in February-March 2006 have been placed in category E as described in the paragraphs below. This study dosed once daily by stomach tube from gestation day 7 through gestation day 19. The dosage levels were 0, 10, 100, 500, 1000, and 2000 mg/kg/day. Possible adverse effects on the approval form included excess salivation, gasping, labored respiration and subdued behavior. The approval states that animal pain/distress can not be relieved and that a plan for addressing animals in pain/distress would be developed at the time of occurrence by the study director and veterinary staff. Based on the expected signs, the vet staff monitored the entire study closely and groups IV-VI (500, 1000 and 2000 MKD) were provided with two water sources and an alfalfa cube daily to help improve feed intake.

1 rabbit #5743 (VI; 2000 MKD) lost 21.5% body weight from GD7-GD20 and was euthanized as scheduled on GD20. This rabbit's feed intake was low from GD7-GD20. This rabbit had two water sources and an alfalfa cube was provided daily. This rabbit was examined four times and remained bright, alert, active and inquisitive. The last of these four exams was in the afternoon the day before scheduled euthanasia. Adverse signs included scant/dry feces and ungroomed coat. This animal has been placed in category E because it can be presumed that pain/distress is associated with a body weight loss of this magnitude and reduced feed intake of this duration.

1 rabbit #5729 (VI; 2000 MKD) was found dead during the morning room check on GD20. This rabbit lost 3.7% body weight from GD7-GD16 then gained 1.2% weight from GD16-GD19 for an overall loss of 2.6%. Feed intake was fair to moderate from GD7-GD20. This rabbit had two water sources and an alfalfa cube was provided daily. Adverse signs included scant/discolored feces. This rabbit was examined three times prior during the monitoring of all animals in the study and was bright, alert and

responsive during exams. Necropsy exam showed stomach erosions, approx. 30 ml of red fluid in the cecum and intestines and nine fetuses in utero. This rabbit has been placed in category E because pain/distress can be presumed to be associated with the adverse findings shown at necropsy and because it is possible that adverse clinical signs associated with pain/distress occurred in the overnight hours prior to death.

1 rabbit #5717 (IV; 500 MKD) was found dead during the morning room check on GD 12. This rabbit lost 4.8% body weight from GD7-GD 11 and feed intake was fair-moderate during this same time period. Adverse signs included soft feces and ungroomed coat. The rabbit was normal at necropsy exam and 12 conceptuses were present in utero. This rabbit has been placed in category E because it is possible that adverse clinical signs associated with pain/distress occurred in the overnight hours prior to death.

Study: #29

Animals: 2 Rabbits (high-dose group)

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.

OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 2 high-dosage rabbits (Group IV; 500 mg/kg/day) from this oral gavage developmental toxicity study have been placed in category E based on the extent and duration of the clinical signs experienced prior to euthanasia. This study was conducted in May 2006 and was approved with expected adverse signs related to test article toxicity including death, body weight loss, reduced feed intake, soft feces and scant feces. The approval states that animal pain and distress can not be relieved. The approvals states that the approved action for addressing rabbits in pain/distress was for the study director and vet staff to decide at the time of occurrence.

1 rabbit #7175 (IV) lost body weight throughout the dosage period from gestation day 7 to 19. This rabbit's feed intake was moderate from gestation day 8 to 12 then low from gestation day 12 to 19. All rabbits in this dosage group and in dosage group III were provided with an additional water source and a timothy hay cube daily beginning on 5/13/06 (gestation day 17 for this rabbit.) This rabbit was examined along with all rabbits in groups III and IV on 5/10/06 and 5/12/06 - 5/16/06 (date of this rabbit's euthanasia.) This rabbit's activity level and appearance remained normal. This rabbit aborted on gestation day 20, the day after the last dosage administration and was euthanized. Body weight loss totaled 16% from gestation day 7 to 19. All conceptuses were resorptions. The rabbit was normal at necropsy exam. This rabbit has been placed in category E acknowledging that the extent and degree of weight loss and low feed intake leading to abortion can be presumed to be associated with pain/distress.

1 rabbit #7179 (IV) lost 15.9% body weight from gestation day 0 until euthanasia on gestation day 14. This rabbit consumed almost no food during the dosage period from gestation day 7 until euthanasia. The vet staff noted this rabbit had a 12% body weight loss while examining all group III and IV animals for expected clinical signs on 5/10/06 (gestation day 13 for this rabbit.) As a result, a second water source and a timothy hay cube were provided at that time. At exam the following day, the rabbit's

behavior was noted as depressed including teeth grinding, dehydration, ptosis and reluctance to move. The rabbit was euthanized at this time. This rabbit has been placed in E because it isn't possible to accurately determine the duration of the behavior changes associated with pain/distress prior to euthanasia. The duration may have been as short as a few minutes or as long as overnight between the two veterinary exams.

Study: #30

Animals: 10 Rabbits

Type of Study: Intravenous Dosage-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 10 rabbits from this study have been placed in category E based on the nature and duration of adverse signs experienced that can be presumed to be associated with pain/distress. This dosage-range finding study was conducted in April 2006. Dosage was by continuous intravenous infusion from gestation day 6 through 19 at 0.43, 4.34 and 21.7 mcg/kg/min. Adverse signs in rats dosed at 8.1 mcg/kg/day included lacrimation, perinasal substance, pale extremities and ptosis. The approval states that animal pain and distress can not be relieved. The approval includes a plan for addressing rabbits experiencing pain/distress on this study by approving the decision to keep dosing, stop dosing or to euthanize to be made by the study director and veterinarian at the time of occurrence. Twenty nine veterinary exams were performed during the conduct of this study.

All animal in groups II and III (**10 rabbits total**) had decreased motor activity, sternal recumbancy, weakness/ataxia (limited use of limbs) or splayed limbs, ocular or nasal discharge, ungroomed coat and reduced fecal output within 24 to 48 hours of the first dosage administration on gestation day 7. It was suspected that these signs were the result of blood pressure lowered by the test article. The decreased motor activity and sternal recumbancy resolved by gestation day 10. The ungroomed coat, reduced fecal output, reduced body weight gain and reduced feed intake generally persisted until the study ended on gestation day 20. Eight of these ten rabbits survived until the day of scheduled sacrifice, one was found dead on gestation day 18 and one was euthanized according to veterinary recommendation on gestation day 18. At necropsy, both the rabbit found dead and the rabbit euthanized by veterinary recommendation had adverse kidney findings, gas-filled intestines and all fetuses were resorbed.

Study: #31

Animals: 2 Rabbits

Type of Study: Oral Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division
- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994.

Rockville (MD).

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.

Diagnosis: 2 rabbits from this developmental toxicity study conducted in May-June 2006 have been placed in category E based on the nature, magnitude and duration of adverse clinical signs and/or necropsy observations. It can be presumed that the nature of these adverse signs and necropsy findings were accompanied by pain/distress. These rabbits were dosed once daily by stomach tube on gestation day 7 through 19. Body weight loss and reduced feed intake were expected at the high dose level (200 mg/kg/day.) The approval states that animal pain and distress can not be relieved. The approval includes a plan for rabbits experiencing pain/distress to be addressed by the study director and vet staff at the time of occurrence.

1 rabbit #7321 (I; 0 mg/kg/day) was discovered aborting during the morning viability checks and was euthanized on gestation day 20. On gestation day 18, this rabbit was examined for 7.9% weight loss beginning on gestation day 15 and low feed intake beginning on gestation day 16. Prior to this, the rabbit had eaten well daily and had gained body weight daily. The rabbit appeared normal on exam and excessive shedding was noted. Two water sources were provided, daily grooming to remove excess hair was initiated and a re-exam was scheduled for gestation day 20. No additional weight loss occurred from gestation day 18 to 19 and feed intake improved from gestation day 18 to 19. Reduced fecal output on gestation day 18 was the only adverse sign noted during this time. At necropsy examination, a large subcutaneous hematoma was present over the ventral thorax and extended into the thoracic cavity. The muscle surrounding the length of the trachea/esophagus also had a hematoma present. It is suspected that this rabbit sustained an accidental injury either during handling for dosage administration or during a rack change on gestation day 15 (first interval of weight loss and reduced feed.) Lab management was informed at the time of necropsy of the suspected injury. Lab management's investigation did not reveal any information that indicated that any staff member had knowledge of accidental injury to this rabbit.

1 rabbit #7346 (IV; 200 MKD) was euthanized per veterinary recommendation on gestation day 28 when inflamed eyelids, ocular discharge, ptosis and discolored eyes were first noted. This rabbit was first examined on gestation day 16 for 1% weight loss from gestation day 15 to 16 and three days of reduced feed intake. The rabbit was otherwise normal and an additional water source was provided along with a timothy hay cube daily. At re-exam on gestation day 18, the rabbit appeared normal, had gained weight from gestation day 16 to 17 and from gestation day 17 to 18 and feed intake had improved to almost 100% over these same intervals. No re-checks were scheduled and two water sources were continued. On gestation day 28 an exam was requested for adverse signs involving the eyes. Rales and lacrimation had been recorded as present since gestation day 26 and cold to touch was present on gestation day 27. An 18.5% body weight loss had occurred beginning on gestation day 22 and feed intake was low beginning on gestation day 24. Euthanasia was performed, the ocular discharge was cultured and blood was collected for serology. Necropsy exam showed seven live fetuses, lung lobes mottled red and tan, a pale area on the heart and red striations on the stomach mucosa and the liver. The culture report was positive for *Pasturella multocida*.

Study: #32

Animals: 3 Rabbits

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 3 pregnant rabbits from this oral (stomach tube) developmental toxicity study conducted in May 2006 have been placed in category E due the nature of their adverse signs (either clinically or at necropsy) and/or for the duration and magnitude of body weight loss. Body weight loss and reduced feed intake were expected at the high dose level (group IV; 10 MKD.) The approval states that animal pain and distress can not be relieved. The plan in the approval for rabbits experiencing pain/distress was for the study director and vet staff to decide at the time of occurrence. Veterinary monitoring was conducted daily for all animals during the dosage period and for all animals in the highest dosage group until all appeared recovered. Thirty four exams were conducted on individual rabbits during the conduct of this study.

1 rabbit #7255 (III) was found dead 11 minutes after dosage administration on gestation day 18. Necropsy findings included lung lobes with multiple red areas of varying sizes. The animal had no adverse signs prior to this incident. This death was considered accidental as the result of dosage error. While death occurred soon after the accidental error, this animal has been placed in category E because the duration can be presumed more than momentary. The technician involved was experienced in the dosage procedure and had no history of problems with this procedure in rabbits. Incidents of this nature are tracked by lab management in order to identify patterns which may indicate the need for re-training of a member of the laboratory staff.

1 rabbit #7268 (IV) lost 19% body weight from gestation day 8 through 23 then gained weight daily until scheduled euthanasia on gestation day 29. Feed intake was low from GD8-GD13, fair from GD13-GD21, moderate from GD21-GD23 and good from GD23-GD29. This rabbit was examined by vet staff daily for 11 days and was provided with two water sources, a timothy cube daily and grooming daily during this time. The rabbit remained alert, active and inquisitive. The only adverse clinical sign was scant fecal output. This animal has been placed in category E because it can be presumed that pain/distress was associated with weight loss of the magnitude experienced prior to the period of daily weight gain.

1 rabbit #7289 (IV) was found dead on gestation day 9 prior to daily dosage administration. No adverse signs were present prior to death. Feed intake was good from gestation day 7 (first dosage) to gestation day 8 and was not recorded from gestation day 8 to death. There was a 13% body weight loss from gestation day 8 to death (dead body weight.) Necropsy findings included numerous black ulcerations in the stomach and nine dead fetuses. This animal has been placed in category E due to probability that the stomach ulcerations resulted in pain/distress prior to death.

Study: #33

Animals: 4 Rabbits

Type of Study: XXX Oral Study of Embryo-Fetal Development in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.

Diagnosis: 4 rabbits from this oral gavage (stomach tube) embryo-fetal developmental toxicity study conducted in March 2006 have been placed in category E based on the nature and duration of adverse signs along with adverse necropsy observations. The approval states that body weight reductions were

expected at the highest dosage level (group IV; 60 mg/kg/day.) The approval states that animal pain and distress can not be relieved. The approval plan for rabbits experiencing pain/distress was for the study director and vet staff to decide at the time of occurrence.

1 rabbit #6315 (group I) died on March 18, 2006 shortly after dosage administration when blood was present from the nose and mouth. Necropsy observations showed pale, spongy lung lobes and white foam and clotted blood in the trachea. It is presumed that this rabbit died as a result of a dosing accident. Laboratory management maintains a tracking system for accidental deaths related to dosing and the course of action is dependent on the staff member's past history of accidental deaths. While death occurred shortly after the dosing accidental, it may be presumed that more than momentary pain/distress was experienced.

1 rabbit #6368 (group IV) aborted eight early resorption on gestation day 20 and was euthanized on March 26, 2006. One lung lobe had a perforation at necropsy. This rabbit was noted with respiratory rales from gestation day 9 through date of euthanasia. This rabbit had several veterinary exams during this time, remained well groomed, alert and active and continued to gain weight with good feed consumption. While the rabbit's condition was good, it is presumed that at some point during the length of time the lung perforation likely existed (approximately 11 days), some pain/distress may have occurred. Lab technical management maintains a tracking system for injuries related to dosing and the course of action taken is dependent on the staff member's past history of accidental injuries.

1 rabbit #6372 (group IV) aborted early resorptions on gestation day 20 and was euthanized on March 27, 2006. All tissues appeared normal at necropsy. This rabbit was noted with respiratory rales on one date, gestation day 8. This rabbit had several veterinary exams beginning at the time rales were first noted. The rabbit remained well groomed, alert and active and gained weight and ate well. While this rabbit's condition was good, it is presumed that the rales on gestation day 8 were the result of some accidental insult to the respiratory tract that may be presumed to be associated with pain/distress. Lab technical management maintains a tracking system for injuries related to dosing and the course of action taken is dependent on the staff member's past history of accidental injuries.

1 rabbit #6388 (group IV) died on March 27, 2006. The lungs were mottled and spongy at necropsy exam. While no adverse signs were noted prior to death, it is presumed that the death was the result of some accidental insult to the respiratory tract during dosage and that this may have been associated with pain/distress prior to death. Lab technical management maintains a tracking system for accidental deaths related to dosing error and the course of action taken is dependent on the staff member's past history of accidental dosing injuries.

Study: #34

Animals: 2 Rabbits

Type of Study: XXX: Thirteen-Day Oral Range-Finding Study in Pregnant Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 2 rabbits on December 11, 2005 were found dead soon after dosage (#4211) or after

attempted dosage (#4249). Blood in trachea, all lung lobes spotted brown. Animals died immediately following dosage accident. The same technician was involved in both instances. The tech was decertified and is no longer employed at CR. While death occurred quickly, it can be presumed that the pain/distress was more than momentary.

Study: #35

Animals: 2 Rabbits

Type of Study: XXX Oral Study of Embryo-Fetal Development in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58

Diagnosis: 2 rabbits from the highest dosage group in this embryo-fetal developmental toxicity study conducted in October 2005 have been placed in category E based on the magnitude and duration of the clinical signs described below. The test article was administered once daily by stomach tube from gestation day 7 through 19. At the time of study approval, information provided indicated that no adverse clinical signs and therefore no more than momentary pain/distress were expected. Following euthanasia of the rabbits described below, this was revised to acknowledge that abortions were expected in the middle and high dosage groups.

2 rabbits #3671 (IV; 80 mg/kg/day) and **#3672** (IV; 80 mg/kg/day) lost 18.4% and 25.4 % body weight respectively over the 13 day dosage period. Little to no feed was consumed over the dosage period. Other adverse signs included scant and/or no fecal output in both rabbits, no viable fetuses detected by palpation and signs of impending abortion for approximately 24 hours (#3671.) On GD17, a veterinary recommendation was made in the morning to euthanize #3671 based on the duration of no feed intake, the magnitude of weight loss and the adverse signs described above. The study director decided to maintain the animal on study at that time and obtained the permission of the IACUC chair by signature on the exam form. The following day, #3671 was re-examined and euthanized. #3672 was euthanized at the time of veterinary recommendation on GD19 when pain/distress was diagnosed based on the duration of no feed intake, the magnitude of weight loss and the adverse signs above. Both rabbits appeared normal at necropsy exam. #3671 had nine live fetuses present and #3672 had seven dead fetuses and one early resorption present.

Study: #36

Animals: 5 Rabbits

Type of Study: Intravenous Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97)

Diagnosis: 5 rabbits from this developmental toxicity study conducted in April-June 2006 have been placed in category E for the reasons described in the paragraphs below. In this study, rabbits were surgically implanted with indwelling vascular access ports and mated *by the supplier* prior to arrival. The surgeries were completed 1-1/2 to 2 weeks prior to arrival. Rabbits were assigned to one of four dosage levels and were dosed by continuous intravenous infusion from gestation day 7 through gestation day 19. No adverse effects related to the test article were expected and unrelated adverse effects included on the approval form were scab on ear, ulceration in vagina, mucoid and/or red substance in cage pan, slight red perinasal substance, ungroomed coat, swelling around port and scant and/or soft feces. The approval states that animal pain and distress can not be relieved. The approval states that the plan for addressing animal pain distress will be decided by the study director and veterinary staff at the time of occurrence.

1 rabbit #6686 (II; 144 mcg/kg/day) was examined on GD13 at 0800. Adverse signs included fur around the mouth and neck wet with saliva, ptosis of both eyes, reluctance to move and grinding of teeth. Feed intake was low during the previous three days and 11% body weight was lost since GD9. Dosage administration was stopped, the jacket/tether system was removed and the rabbit was provided with a second water source, a timothy cube and an empty bowl as an additional toy. At 1330, the rabbit had consumed some of the timothy cube, some pelleted feed and was bright, alert, inquisitive and well-groomed. Soft feces was present so daily cleaning of the floor grate was prescribed. At 0750 on GD14, the rabbit had gained 5.4 % weight in 24 hours, had consumed 100% of the feed provided and was bright, alert, inquisitive and observed playing with the toy. Dosage administration was resumed. At exam on GD 15 and until scheduled euthanasia on GD22, this rabbit gained weight, ate well and appeared normal including fecal consistency. This rabbit is being placed in category E acknowledging that the adverse clinical signs associated with pain/distress may have been present for some period of time in the overnight hours prior to discovery on the morning of GD13.

1 rabbit #6643 (III; 432 mcg/kg/day) sustained a tear in the left ear approximately 2.0 cm in length where the ear tag had previously been. The area was rinsed with hydrogen peroxide and the cage interior was cleaned. At re-exam the next day, a dry scab was present. There was no redness and no swelling but the rabbit appeared apprehensive and resisted touching of the ear. At re-exam the following day, the rabbit behaved normally and was not sensitive to approach or to touching of the ear. No adverse findings were present for the remainder of the study and this rabbit was euthanized as scheduled. This rabbit has been placed in category E due to the pain/distress apparent on the day following the injury to the ear.

1 rabbit #6662 (IV; 1440 mcg/kg/day) had a scab approximately 2.0 x 0.5 cm present on the right ear when veterinary exams were done on the day of arrival. There was no indication of pain or distress when the ear was examined. When clinical observations were next performed on GD6, the scab was no longer present. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the inflammatory process leading to formation of a scab of this size at some point in time.

1 rabbit #6615 (I; 0 mcg/kg/day) had a scab on the back approximately 2.5 cm in diameter present from GD24 to GD29. The scab and surrounding tissue appeared healthy on exam and there was no apparent pain/distress associated with handling of the area. The body weight increased daily during this time. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the inflammatory process leading to formation of a scab of this size at some point in time.

1 rabbit #6618 (I; 0 mcg/kg/day) had a scab on the back approximately 1.5 cm in diameter present from GD21 to GD29. The scab and surrounding tissue appeared healthy on exam and there was no apparent

pain/distress associated with handling of the area. The body weight increased daily during this time. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the inflammatory process leading to formation of a scab of this size at some point in time.

Study: #37

Animals: 6 Rabbits

Type of Study: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits (Non-GLP)

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 6 rabbits from this dosage range-finding study conducted in June 2006 have been placed in category E based on the nature and duration of adverse clinical signs and the nature of the adverse necropsy observations. These adverse signs can be presumed to have been associated with pain/distress. Rabbits were dosed by stomach tube from gestation day 7 through 19 at dosage levels of 0, 50, 200, 600 and 1,000 mg/kg/day. 600 mg/kg/day caused death in rats. This test article acted on the blood and blood-forming organs and had not been given to rabbits before. Expected adverse signs included death, dehydration, abdominal distension, coldness, loss of righting reflex, salivation, decreased fecal output, decreased feed intake, body weight loss and labored breathing. The study was approved with moribund condition as an endpoint. The rabbits were checked hourly until the end of the work day after daily dosage administration. Animals were also monitored frequently by the veterinary staff

1 rabbit #7618 (IV; 600 MKD) showed decreased motor activity after first dosage on gestation day 7 and was found dead by the vet staff on the following morning. (All remaining animals appeared normal at this time.) This rabbit lost no body weight. Feed intake was low from GD7-GD8. Necropsy examination showed black areas on the thymus and a red trachea.

1 rabbit #7617 (IV; 600 MKD) was found dead by study staff on GD8 just prior to the second dosage administration. This rabbit had an ungroomed coat and perioral substance after the first dosage on GD7. This rabbit had no body weight loss. Feed intake was low from GD7-GD8. Necropsy examination showed red perioral substance, black areas on the thymus and on the lungs and a blue trachea.

1 rabbit #7621 (V; 1000 MKD) died during veterinary evaluation at 1440 approximately one hour after the second dosage administration on GD8. This rabbit appeared normal prior to dosage on GD8. Feed intake was low from GD7-GD8. At veterinary examination, this rabbit was alert when handled but otherwise uninterested in the surroundings and appeared dull and listless. The rabbit died immediately upon becoming laterally recumbent during the exam. Necropsy exam showed a red trachea, fluid inside the pericardial sac, red fluid inside the intestines and inflamed stomach mucosal surface. At this same time on GD8 (1440, approximately one hour after dosage), the vet staff also noted that all remaining rabbits in the 600 and 1000 MKD groups appeared dull and listless and therefore recommended that the animals in these groups be euthanized.

1 rabbit #7620 (IV; 600 MKD) died approximately 4.5 hours after the second dosage administration on GD8. This rabbit appeared normal on GD7, had no body weight loss and feed intake was low from

GD7-GD8. Adverse clinical signs after the second dosage included ptosis, rapid respiration and decreased motor activity. Necropsy exam showed red areas in the subcutaneous skin of the chest and abdomen, a red trachea and stomach erosions.

Note. As a result of veterinary recommendation at 1440 on GD8 and the mortality after only one or two doses, all remaining rabbits in the 600 MKD group (2 rabbits) and all remaining rabbits in the 1000 MKD group (4 rabbits) were euthanized in the early evening hours of GD8 and have been reported in category D.

1 rabbit #7615 (III; 200 MKD) was found dead during the morning viability checks on GD 12. No adverse clinical signs were noted at any time prior to being found dead. This rabbit lost 2.7% body weight from GD10-GD11. Feed intake was good until GD10-GD11 when intake was moderate. Necropsy examination showed a red trachea and red areas on the thymus.

1 rabbit #7614 (III; 200 MKD) was found dead at morning viability checks on GD14. Hyperpnea was noted on GD 12 and scant feces on GD 13. There was 6% body weight loss from GD8-GD13 and low feed intake during the same period. Necropsy examination showed red areas on the thymus.

Study: #38

Animals: 2 Rabbits (1 high-dose; 1 mid-dose)

Type of Study: Intravenous Dosage-Range Developmental Toxicity Study of XXX in Female Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 2 rabbits from Part A of this study conducted in December 2005 were placed in category E because it may be presumed that the acute toxicity resulting in reduced body weight, feed intake and lung pathology may have also caused pain/distress in the overnight hours prior to death. Part A of this dosage-range developmental toxicity study was designed to provide information on the ability of non-pregnant rabbits to tolerate repeated daily intravenous doses of the test article. The study was approved to result in pain/distress and possible mortality from toxicity. The approval states that animal pain and distress can not be relieved. The approvals states that the plan for addressing animals in pain/distress will be decided by the study director and veterinary staff at the time of occurrence.

1 rabbit #4341 (high dose) was found dead in the morning of the day following the first day of dosage administration. All lung lobes contained dark red foci and the rabbit was otherwise normal at necropsy. The only adverse clinical sign noted on the day of the first dosage was a white periocular substance at the check one hour after dosage administration. The body weight at necropsy was less than the previous day's weight and feed consumption was also lower. Note: All remaining rabbits in the high dosage group were euthanized on day 2 of dosing (the same day #4341 was found dead) and the high dosage level selected for Part B of this study was one-third of the high dosage level used in Part A.

1 rabbit #4338 (mid-dose) was found dead in the morning of the day following the second day of dosage administration. All lung lobes contained dark red to black foci and the thymus was mottled red to dark red. Adverse clinical signs noted on the day prior to death included an ungroomed coat,

decreased activity and vocalization for approximately ten seconds during the 1 hour dosage administration. Body weight and feed intake were also reduced following the first dosage administration. Note: All remaining animals in this group were euthanized on the same day #4338 was found dead and the mid-dosage levels selected for Part B of this study were 10% and 30% of the mid-dosage levels used in Part A.

Study: #39

Animals: 5 Rabbits (high-dose group)

Type of Study: Intravenous Developmental Toxicity Study of XXX in Female Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 5 high-dosage group rabbits have been placed in category E based on the nature and duration of the adverse clinical signs and the nature of the necropsy observations. It can be presumed that these adverse findings were associated with pain/distress signs and resulted in death. This was an intravenous developmental toxicity study in pregnant rabbits conducted in March-April 2006. The dosage levels selected for this study were 0, 10, 20 and 40 mg/kg/day based on a previous study conducted at this facility. In that study (PHX00001), one rabbit at the 40 mg/kg/day level was found dead on gestation day 9 attributed to the test article and all remaining animals at two higher dosage levels were terminated due to adverse signs/mortality. The potential adverse test article effects included on the approval form for PHX00002 were periocular substance, decreased motor activity, ungroomed coat, scant feces, body weight reduction, and reduced feed intake. The approval states that animal pain and distress can not be relieved. The action plan approved for animals experiencing pain and distress was for the study director, in consultation with the veterinarian, to determine if administration will be halted, animals euthanized or, in support of the study objectives and/or regulatory requirements of the study, animals be continued

On March 22, 2006, 2 high dose **rabbits** (40 mg/kg/day) were found dead on gestation day 9. Each had minor body weight loss and low feed intake from gestation day 7 (first date of dosage) to death. Necropsy exams showed large kidneys in both rabbits.

On March 25, 2006, 3 high dose **rabbits** (40 mg/kg/day) were found dead on gestation day 9. Two of these rabbits (#6278 and #6279) had white periocular substance observed on gestation day 7 and lost minor weight and had minor feed intake reduction from gestation day 7 to death. #6279 was examined the day prior to death and was noted to have a decreased activity level. A second water source was recommended and provided and a re-check exam was scheduled. At necropsy, both of these rabbits had large kidneys and mottled lungs. The third rabbit (#6280) also lost a minor amount of weight and had low feed intake gestation day 7 to death. No other adverse signs were noted prior to death. The kidneys were large and the thymus was mottled at necropsy.

Note: Subsequent exams of the remaining high dosage (40 mg/kg/day) animals revealed no major weight reductions or feed reductions and no adverse signs indicative of pain/distress.

Study: #40

Animals: 19 Hamsters

Type of Study: Subcutaneous, Single-Dose Study of XXX Oncogenic Potential in Neonatal Goden Syrian hamsters [1-3 Day Old Hamsters (Neonates) with an Approximately Five Month Observation Period

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].
- U.S. Food and Drug Administration (1993). Points to consider in the characterization of cell lines used to produce biologicals
- European Pharmacopoeia Monograph 5.2.3, Cell substrates for production of vaccines for human use. 01/2005:50203

Diagnosis: 19 hamsters from this study evaluating the oncogenic potential of a vaccine have been placed in category E as described in the paragraphs below. This study was conducted in February 2006 through August 2006. At or soon after birth, hamsters were given subcutaneous injections at two sites (0.1 ml per site) and then monitored for approximately 5-1/2 months for the possible formation of masses. The animals remained with their dams until weaning and were then housed singly in nesting boxes containing bedding and nesting materials. Animals were fed *ad lib* and no feed consumption data was collected. Nylabones® were provided for chewing activity. Water was provided by water bottles and the sippers were checked twice daily for proper functioning. Veterinary staff monitored the animals daily for the first 2-1/2 months of the study and weekly thereafter through the end of the study. The study was approved with no more than momentary pain/distress expected from composition of the test article acknowledging that more than momentary pain/distress could result from the injection volume of 0.1 ml per site. Approval also acknowledged that the growth of masses could result in more than momentary pain/distress.

3 hamsters #7702 (group II, male), **#7908** (group I, female) and **#7705** (group II, female) were found dead within one to seven days following dosage administration. No adverse clinical signs or weight loss were noted prior to death. Following the instruction of the protocol, no necropsy was performed and the carcasses were discarded. These neonates have been placed in category E acknowledging that it is possible that more than momentary pain/distress was experienced prior to death.

1 hamster #7804 (group II, male) was euthanized at seven days of age by veterinary recommendation as the result of moribund condition. This pup was dosed on day one of age and appeared normal at dosage and at one hour after dosage. At four hours after dosage, a small scab (0.1 x 0.2 cm) was noted on the right flank presumably a result of the dam. No adverse signs were noted during the morning check of the study animals on day seven of this pup's life. At the time of the vet exam in the late afternoon of this same day, the pup was lethargic, extremely dehydrated, had no milk visible in the stomach and the abdomen appeared distended. The pup was euthanized. Necropsy exam revealed that the intestines were distended with gas. This animal has been placed in category E because the clinical signs present can be presumed to be associated with pain/distress and this pain/distress may have been more than momentary since there is no way of accurately knowing the time of onset following the morning room check in relation to the time of discovery later that afternoon.

14 hamsters (#8202, #8003, #8303, #8304, #8504, #7905, #8106, #8208, #8307, #8407, #7706, #7807, #7907, #8107) had swelling and/or discoloration (purple) at an injection site noted on day 1 of age, the

day of the injection. Clinical signs were recorded weekly and these adverse signs were no longer present when clinical signs were next recorded on day 8 of age. From day 1 to day 8 of age, these pups gained body weight at a rate comparable to other pups of the same age and sex indicating the ability to successfully nurse and the receipt of proper care from the dam. These hamsters have been placed in category E acknowledging that while there is no clear evidence that more than momentary pain/distress was experienced on the day of injection, it is possible that more than momentary pain/distress was experienced if these signs persisted for an extended period of time before being noted as resolved on day 8 of age.

1 hamster #7707 (group I, female) gained body weight weekly throughout the study then lost 2.3% body weight between day 134 and day 141 of the study and an additional 14.8% between day 141 and day 146, the day of scheduled euthanasia. This is a total loss of 17.1 % over 12 days. No other adverse signs were present. All tissues were normal at necropsy examination. On each day of the study, water sippers were checked twice daily for proper functioning and cageside observations including an assessment for adequate fecal output were done at least twice daily. This animal has been placed in category E acknowledging that body weight loss of the magnitude experienced could itself be considered painful/distressful or possibly be indicative of the presence of some other painful/distressful condition.

Study: #41

Animals: 5 Hamsters

Type of Study: Subcutaneous, Single-Dose Study of XXX Cell Lysate Oncogenic Potential in Neonatal Golden Syrian Hamsters [1-3 Day Old Hamsters (Neonatal)] with an Approximately Five Month Observation Period

Guidelines/Regulations:

- These studies are not formally recognized in published guidelines; the relevant regulatory references are included in the protocol. The Sponsor has been directed by the regulatory agencies to use this model and study design to investigate the safety of this vaccine.

Diagnosis: 5 hamsters from this oncogenic potential study conducted in March 2006-August 2006, have been placed in category E as described in the paragraphs below. At or soon after birth, hamsters were given subcutaneous injections (two sites, 0.1 ml per site) and then monitored for approximately 5-1/2 months for the possible formation of masses.

The animals were palpated weekly for masses and body weights and clinical observations were performed weekly. Animals were fed *ad lib* and no feed consumption data was collected. Water was provided by water bottles and the sippers were checked twice daily for proper functioning. The animals remained with their dams until weaning and were then housed singly in nesting boxes containing bedding and nesting materials. Nylabones were provided for chewing activity. Veterinary staff monitored the animals daily for the first sixty days of the study and weekly thereafter through the end of the study. The study was approved with no more than momentary pain/distress expected from composition of the test article acknowledging that more than momentary pain/distress could result from the injection volume of 0.1 ml per site. Approval also acknowledged that the growth of masses could result in more than momentary pain/distress.

1 hamster #3005 (group II, female) had limited use of the right front paw and leg from day 8 of age through day 15 of age and was normal from day 22 of age through the end of the study. During the period of limited use of the limb, this animal gained body weight at a rate comparable to other female pups of the same age indicating the ability to successfully nurse and the receipt of proper care from the dam. This hamster has been placed in category E acknowledging that while there is no clear evidence that more than momentary pain/distress was experienced, it is possible that the limited use of the limb did result in more than momentary pain/distress.

1 hamster #3404 (group I male) lost small amounts of body weight (less than 5%) weekly for seven consecutive weeks leading up to the scheduled end of the study for a total loss of 16.1%. No other adverse signs were present. On each day of the study, water sippers were checked twice daily for proper functioning and cage side observations including an assessment for adequate fecal output were done at least twice daily. This hamster has been placed in category E acknowledging that body weight loss of the magnitude experienced could itself be considered painful/distressful or possibly indicative of the presence of some other painful/distressful condition.

3 hamsters #3307 (group I female), **#3408** (group I female) and **#3203** (group I male) had swelling and/or discoloration (purple) at an injection site noted on day 1 of age, the day of the subcutaneous injections. These signs were no longer present when clinical signs were next recorded at day 8 of age. From day 1 to day 8 of age, these pups gained body weight at a rate comparable to other pups of the same age and sex indicating the ability to successfully nurse and the receipt of proper care from the dam. These hamsters have been placed in category E acknowledging that while there is no clear evidence that more than momentary pain/distress was experienced, it is possible that the swelling or discoloration did result in more than momentary pain/distress.

Study: #42

Animals: 6 Rabbits

Type of Study: Oral (Stomach Tube) Dosage-Range Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- This is a dosage-range study for dosage selection for future studies that will be based on U.S. Department of Health and Human Services Food and Drug Administration: Guidelines for Industry: detection of toxicity to reproduction for medicinal products, (ICH) S5A; September 1994, Rockville MD. These guidelines require that dosages produce some maternal toxicity. Conducting the dosage range study prior to a full study, allows the determination of a toxic dose acceptable for use while only using a few animals. Without the conduct of this dosage-range study prior to required full developmental toxicity studies, larger numbers of animals may be needed for the full study.

Diagnosis: 6 rabbits from this dosage-range developmental toxicity study conducted in April-May 2006 have been placed in category E for the reasons described in the paragraphs below. The highest dosage level was expected to cause mydriasis, body weight loss, low feed intake, hyperactivity, salivation, tremors, fast respiration and mortality. The approval states that animal pain and distress can not be relieved. The approved plan for rabbits experiencing pain/distress was for the study director and veterinarian to decide what to do (continue on study, stop dosing or euthanize) at the time of occurrence.

1 rabbit #7071 (group IV; high dose) had low feed intake and body weight loss from GD8 (the day following the first dosage) until unscheduled euthanasia on GD20. The animal was examined by veterinary staff for weight loss and was provided with a second water source and a timothy cube in an effort to encourage improved feed intake. At re-examination the following day, pain/distress was diagnosed due to the duration and severity of the reduced feed intake and weight loss (19.3% from GD8 to GD20.) The animal was euthanized as the result of the examination. The rabbit appeared normal at necropsy evaluation. All conceptuses were resorption. This animal has been placed in category E due to the duration and the severity of the reduced feed intake and weight loss prior to unscheduled euthanasia.

1 rabbit #7072 (group IV; high dose) experienced tachypnea on GD8 and hyperpnea on GD11. Feed intake was low to moderate during the dosage period from GD7 to GD19 and returned to normal when the dosage period ended. This rabbit lost 19% body weight during the dosage period then gained weight daily when the dosage period ended. This rabbit gained 18% body weight in the post dosage period

prior to scheduled caesarean-sectioning on GD 29. This rabbits has been placed in category E because pain/distress may have been associated with low feed intake and weight loss of the magnitude and duration noted prior to the period of improved feed intake and weight gain.

1 rabbit #7073 (group IV; high dose) had low feed intake and body weight loss from GD8 (the day following the first dosage) until death on GD18. This rabbit lost 16% body weight from GD7 to GD 17 and was found dead in the morning on GD18. This rabbit appeared normal on necropsy exam. All conceptuses were resorptions. This animal has been placed in category E due to the duration and the severity of the reduced feed intake and weight loss over a ten day period.

1 rabbit #7075 (group IV; high dose) received test article on GD7 and GD8 prior to being found dead in the morning of GD9. No adverse clinical signs were present on prior to death. The rabbit lost 4.5 % body weight from GD 7 to GD 8 and had low feed intake during this same time. This rabbit was normal at necropsy and was pregnant with seven embryos. There is no way to know whether or not this rabbit experienced pain/distress prior to death. Considering that at least four other rabbits at this same dosage group experienced adverse respiratory signs (tachypnea, hyperpnea) on GD 8, it is reasonable to presume this rabbit may have also experienced similar effects or some other effect associated with more than momentary pain/distress prior to death.

1 rabbit #6548 (group IV; high dose, satellite) experienced tachypnea and head bobbing on GD11 through GD13. This rabbit lost 3.6 % body weight from GD8 to GD11 then maintained weight GD 11 to GD 14. Feed intake was moderate from GD8 to GD 12 then low on GD13. This rabbit was found dead in the morning on GD 14. There were black areas on the thymus at necropsy examination. Eleven embryos were present. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with the adverse respiratory signs noted on GD11 through GD13.

1 rabbit #6550 (group IV; high dose, satellite) aborted and was euthanized on GD 18. Prior to abortion, this rabbit lost 19% body weight from GD7 to GD 17 and had low feed intake during this same time period. This rabbit was normal at necropsy examination. Eleven embryos were present. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with low fed intake and weight loss of the duration and magnitude experienced prior to euthanasia for abortion.

Study: #46

Animals: 2 Rabbits

Type of Study: XXX: Embryo-Fetal Toxicity Study in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 2 rabbits from this stomach tube embryo toxicity study conducted in June 2006 have been placed in category E based on the presence of adverse clinical and/or necropsy signs. The nature of these adverse signs can be presumed to have been associated with pain/distress. Expected adverse test article effects included body weight loss and reduced feed intake at the 60 mg/kg/day level and

mydriasis at the 5, 30 and 60 mg/kg/day levels. The approval states that animal pain and distress can not be relieved. The approval states that timothy cubes can be provided to rabbits with low feed intake. The approval states that moribund condition is the endpoint.

1 rabbit #7769 (IV; 60 MKD) was noted with moderate feed intake of a two day duration during veterinary monitoring of the study for expected feed and weight reduction at this dosage level on GD9. The rabbit was bright, alert and active and otherwise normal. A timothy cube was provided daily and daily grooming was initiated to minimize ingestion of loose hair. Animal was examined again on GD 12, GD 13, GD 14 and GD 16. The rabbit remained bright, alert, active and otherwise normal. Feed intake remained low and timothy cube intake also appeared low. When the cumulative body weight loss reached 15% at exam on GD16, pain/distress was documented based on the magnitude of weight loss and duration of low feed intake over a nine day period. A re-check was scheduled for GD 17. This rabbit was found to have aborted during the morning check on GD 17 and was euthanized. The rabbit was normal at necropsy exam. All uterine contents were early resorption.

1 rabbit #7776 (IV; 60 MKD) was euthanized by veterinary recommendation on GD7 for adverse signs suggestive of an accidental injury to the respiratory tract during dosage administration on GD6. At dosing on GD6, the stomach tube was inserted several times prior to dosage administration. Respiratory rales were noted on GD 7, there was an 8% body weight loss in 24 hours and feed intake was reduced to a low level. Necropsy exam showed dark red apical lung lobes on the left and right. The technician is experienced in dosage by stomach tube and had no history of problems with this procedure so no action was taken. Lab management maintains a tracking system of accidental injuries to identify the need for re-training of any laboratory staff member.

Study: #44

Animals: 12 Rabbits

Type of Study: Oral (Stomach Tube) Single-Dose Pharmacokinetic Study of XXX in Male and Female Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58; last revised April 1, 2002; U.S. Federal Government Archives.
- U.S. Dept of health and Human Services Food and Drug Administration. Guideline for Industry; toxicokinetics: the assessment of systemic exposure in toxicity studies, ICHS3A; March, 1995, Rockville (MD).

Diagnosis: 12 rabbits have been placed in category E for adverse clinical and/or necropsy signs that can be presumed to be associated with pain/distress. This was a single dose oral gavage (stomach tube) pharmacokinetic study in male and female rabbits conducted in March 2006. There were three males and three females assigned to each of four dosage groups for a total of 24 rabbits. The study was approved with expected signs including tremors, rapid respiration, salivation, hyperactivity and mortality. The approval states that animal pain and distress can not be relieved. The approval states that the plan for rabbits experiencing pain/distress is for the study director and vet staff to decide at the time of occurrence. The animals were dosed in the morning at approximately 0830 and blood collections were done on all animals at the following intervals after dosage: 15 min., 30 min., 1 hr., 2 hr., 4 hr., 6 hr., 8 hr., 12 hr. and 24 hr. Animals exhibiting clinical signs were examined by a staff veterinarian and all animals were observed daily by veterinary staff until the end of the study.

All **3 male rabbits** in each of the 120, 180 and 240 mg/kg/day dosage groups (total of 9 rabbits) were found dead (**7 rabbits**) or euthanized on day 1 or 2 of the study (**2 rabbits**.) Observations of mydriasis and hyperpnea occurred in all the rabbits placed in category E. Hyperactivity, tremors, pale appearance and moderate excess salivation occurred in single rabbits in the 180 and 240 mg/kg/day dosage groups

and ataxia occurred in a single rabbit in the 240 mg/kg/day dosage group. Observations of decreased motor activity, hyperactivity, vocalization, circling, miosis, cold to touch and extreme excess salivation and blue mucous membranes occurred in a non-dosage dependent manner in single rabbits in the 120, 180 and/or 240 mg/kg/day dosage groups. Necropsy observations in these rabbits included discolored thymuses, stomach ulcerations and tan areas on the liver and kidneys.

All **3 female rabbits** in the 240 mg/kg/day dosage group were found dead on day 1 or day 2 of the study. Mydriasis was observed in all three female rabbits placed in category E. Tremors, moderate excess salivation, hyperpnea, gasping and soft or liquid feces occurred in a single rabbit in category E. Necropsy observations for these rabbits were discolored thymuses and one rabbit had a diaphragmatic hernia (portion of left lateral liver lobe and stomach protruded into the thoracic cavity.)

Study: #45

Animals: 2 Rabbits (mid-dose group)

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis:

1 female rabbit #6752 (III; mid-dosage level) was euthanized upon veterinary examination on day 12 of presumed gestation due to adverse clinical signs. This rabbit was dosed orally by stomach tube once daily for six days prior to euthanasia. On the day prior to euthanasia, rales were noted in this rabbit. Feed consumption was reduced from gestation day 11 to 12 (day of euthanasia) by 50%. There was no body weight loss. The rabbit's posture at examination indicated pain and distress and when approached the respiratory sounds increased. Necropsy examination revealed that the rabbit apparently received some test article in the lungs probably a day prior to euthanasia. Lab management maintains a tracking system of accidental injuries in order to identify the need for re-training of any lab personnel. This rabbit has been placed in category E because it can be presumed that pain/distress was associated with an injury of this nature existing for at least one day prior to euthanasia.

1 female rabbit #6788 (III; mid-dosage level, Satellite) was examined for 14% body weight loss and low feed intake during the dosage period. These effects were approved as expected test article effects at this dosage level. The approval states that animal pain and distress can not be relieved. The approval states that the plan for addressing animals in pain/distress is for the study director and veterinary staff to decide at the time of occurrence. The rabbit was alert and active but also noted as apprehensive which may have indicated pain or distress. Treatment was initiated. An alfalfa cube was provided daily to help improve feed intake and grooming was done daily to minimize hair ingested by the rabbit while feed intake was low. On re-examination, there was no additional body weight loss but the feed intake remained low and the animal remained apprehensive. Treatment continued. At the next re-examination, the rabbit was eating well, gaining weight and no longer appeared apprehensive. The rabbit continued to do well and was euthanized as scheduled at the end of the study. This rabbit has been placed in category E because apprehension can be presumed to be an indication of pain/distress and weight loss of the

Study: #46

Animals: 1 Rabbit

Type of Study: Oral (Stomach Tube) Developmental Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis:

1 rabbit #3861 (group III Satellite; gestation day 18) was normal for the entire study prior to death immediately following intubation by stomach tube for test article administration on 10/23/05. Necropsy examination revealed a torn lung lobe. Lab management reviewed the circumstances of this accidental death. The technician involved had been certified on performing this procedure since 8/14/04 and this was the first time this technician had a dosage accident. While only a very brief period elapsed between the accident and death, there is no way of knowing for certain to what degree, if any, the animal experienced pain and/or distress. Consequently, the animal is being reported in category E.

Study: #47

Animals: 5 Rabbits (high-dose group)

Type of Study: Developmental Toxicity Study on the Effects of Intravenous Infusion of XXX on Embryo-Fetal Toxicity in Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58
- Japanese Ministry of Health, Labour and Welfare (1997). Good Laboratory Practice Standard for Safety Studies on Drugs, MHW Ordinance Number 21, March 26, 1997.
- Organisation for Economic Co-operation and Development (1998). The Revised OECD Principles of Good Laboratory Practices [C(97) 186/Final].

Diagnosis: 5 rabbits in the highest dosage group (V) from this intravenous developmental toxicity study conducted in January 2006 have been placed in category E because pain/distress can be presumed to have been associated with clinical signs including weight loss and/or necropsy observations of the magnitude and duration described below. This study was approved to cause no more than momentary pain/distress due to test article effects.

1 rabbit #5382 (group V) was euthanized at the time of veterinary re-examination. This animal began losing weight and had reduced feed intake five days prior to euthanasia. Weight loss at that time was 3% and the animal was otherwise normal in behavior and appearance. Two water sources were provided and the animal was groomed daily to minimize the ingestion of loose hair during grooming while feed intake was low. On re-exam, the weight loss total was 16%, feed intake remained low and

signs of impending abortion were present. The rabbit's attitude and behavior remained normal. At necropsy, the rabbit was normal and all conceptuses were resorptions.

1 rabbit #5384 (group V) was euthanized as scheduled according to the study design. At exam the day before, the rabbit had a four day history of reduced feed intake and had lost body weight for six days. The amount of weight loss was inadvertently incorrectly calculated at the time of exam resulting in a value that appeared minor when it was not. The rabbit's behavior was normal, the fur was well groomed and there was low fecal output. By the next day (the day of scheduled euthanasia), the weight loss was 18% over seven days. The rabbit was normal at scheduled necropsy exam and all fetuses were alive.

1 rabbit #5387 (group V) was found dead on gestation day 26. Three days before at veterinary exam, this rabbit had lost 10% weight over three days, had reduced feed intake and was otherwise normal. Two water sources were provided, an alfalfa cube was provided daily and daily grooming was begun. At re-exam the next day, there was an additional 3% weight loss and the rabbit remained otherwise normal. No additional adverse observations were noted for this rabbit prior to death. Total weight loss at death was 14% over seven days. Necropsy showed dark kidneys, a mottled liver and dead fetuses.

1 rabbit #5397 (group V) was found dead on gestation day 22. Earlier this same day, this rabbit was examined for a one-day weight loss of 9%. The rabbit appeared otherwise normal on exam and was provided with two water sources and an alfalfa cube. Later this same day, the rabbit was found dead. Necropsy showed a dark spleen and all fetuses were either dead or resorbed.

1 rabbit #5398 (group V) was euthanized on gestation day 19 at the time of veterinary re-exam for dehydration, loose feces and 21% weight loss over six days.. This rabbit was first examined on gestation day 14 for a one-day minor weight loss. Two water sources and a daily alfalfa cube were provided. At re-exam on gestation day 17, the weight loss was 16% over four days and the rabbit was well-groomed and otherwise normal.

Study: #48

Animals: 2 Rabbits (high-dose group)

Type of Study: XXX Subcutaneous Embryofetal Developmental Toxicity Study in Rabbits

Guidelines/Regulations:

- U.S. Dept of Health and Human Services Food and Drug Administration. Guideline for Industry; detection of toxicity to reproduction for medicinal products, (ICH) S5A; September, 1994. Rockville (MD).
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58
- Pharmaceutical Affairs Bureau, Ministry of Health, Labour and Welfare, GLP standard ordinance for non-clinical laboratory studies on safety of drugs, MHW Ordinance No. 21; March 26, 1997. Japan.

Diagnosis: 2 rabbits from the highest dosage group (20 mg/kg) in this subcutaneous embryofetal developmental toxicity study conducted in February-March 2006 have been placed in category E. The magnitude and duration of body weight loss, duration of reduced feed intake and accompanying adverse clinical signs are the reasons for placement in category E. The rabbits were dosed every other day from gestation day 7 through gestation day 19 and the study ended with euthanasia on gestation day 29. The study was approved with the knowledge that the test article could cause injection site lesions, increased reticulocyte counts and increased hemoglobin. The approval states that animal pain and distress can not be relieved. The approval states that the plan for rabbits experiencing pain/distress is for the study director and veterinary staff to decide the course of action (continue on study, stop or reduce dosage, or

euthanize) at the time of occurrence.

1 rabbit #5970 (20 mg/kg) aborted and was euthanized on gestation day 27. The rabbit was normal until gestation day 17 when feed intake was reduced. The animal received a second water source at that time. The rabbit began to lose body weight on gestation day 21 and lost 20% from gestation day 21 to 26. Mucoid feces was noted on gestation day 26. This rabbit was normal at necropsy exam and all fetuses were dead or cannibalized.

1 rabbit #5976 (20 mg/kg) was euthanized on gestation day 27. This rabbit began to lose weight on gestation day 15 and received a second water source at that time. The rabbit lost 22% body weight from gestation day 15 to gestation day 27 and had reduced feed intake from gestation day 18 to gestation day 27. This rabbit was examined the day before euthanasia with 15% weight loss, mucoid feces and reduced activity level. The next day based on re-exam, the rabbit was not improved and was euthanized.

Study: #49

Animals: 2 Rabbits

Type of Study: Oral (Stomach tube) Developmental Toxicity Study of XXX in Rabbits, Including a Toxicokinetic Evaluation

Guidelines/Regulations:

- U.S. Food and Drug Administration (1994). International Conference on Harmonization; Guideline on detection of toxicity to reproduction for medicinal products. *Federal Register*, September 22, 1994, Vol. 59, No. 183.
- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58
- OECD Environment Directorate. OECD Principles of good laboratory practices, [C(97) 186/Final] (1998); Environmental Health and Safety Division

Diagnosis: 2 Rabbits from this developmental toxicity study conducted in September 2006 have been placed in category E as described in the paragraphs below. Reduced feed intake was an expected test article effect.

1 rabbit (1 mg base/kg/day) was found dead immediately after dosage administration by stomach tube on gestation day 16. This rabbit reportedly struggled during dosage administration then began gasping and died 5 minutes later. This rabbit gained weight and ate well daily up until the time of death. Necropsy examination showed a perforation of one lung lobe and tan mottled areas on each of the lung lobes located on the right. Lab management maintains a tracking system of accidental deaths in order to help identify trends related to any particular staff member. This rabbit has been placed in category E acknowledging that the pain/distress experienced in the five minute period from dosage administration until death could be considered more than momentary.

1 Rabbit (3 mg base/kg/day) consumed an increasingly reduced amount of feed daily beginning on GD7, the first day of dosage, until GD13 when intake became essentially nothing through GD19. When dosage period ended on GD19, feed intake returned to a good level through the end of the study. Five exams were conducted during the dosage period. At all exams, the rabbit remained bright, alert, responsive and inquisitive. Beginning on GD 13, the rabbit ate 3 timothy cubes daily. The maximum body weight lost was 11.3% and all weight was regained by GD23. The timothy cube treatment was stopped on GD23. This rabbit has been placed in category E because it can be presumed that 7 days of low to no feed consumption in a pregnant rabbit is indicative of more than momentary pain/distress.

Study: #50

Animals: 6 Rabbits

Type of Study: Maintenance & Training Protocol for Testing Facility General Population Rabbits

Guidelines/Regulations:

- U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58 – GLPs require that personnel are adequately trained to perform technical procedures necessary to conduct a studies that are conducted according to these regulations.

Diagnosis: 6 rabbits have been placed in category E as described in the paragraphs below. This rabbit colony is maintained in-house for the purpose of providing rabbits for use in training the technical staff in various procedures. The IACUC reviews and approves the protocol annually.

2 Rabbits #5216 and #5428 had scabs (3.0 x 2.0 cm and 1.0 x 1.0 cm, respectively) present on one ear shortly after completion of a five day intravenous testing procedure using 30% ethanol. Neither rabbit experienced a reduction in feed consumption or body weight during this time. A supervisor in the Training group checked the ears daily during the IV testing and swelling and/or discoloration was noted. The supervisor also noted that no pain or discomfort was apparent when the ears were handled. #5216 was euthanized two days after the scab was first noted when several animals were euthanized in order to reduce the number of animals in the training colony. The ear of #5428 was healed (scab no longer present) 6 days after it was first noted and the animal was euthanized later that month when several animals were euthanized in order to reduce the number of animals in the training colony. These rabbits have been placed in category E acknowledging that the tissue reaction resulting in the formation of scabs of this size at the site of ethanol injection may have been associated with more than momentary pain/distress as some point in time.

1 rabbit #5218 had a 4.0 x 0.3 cm tear of the right ear where the ear tag was previously located. There was no bleeding at the time of exam and no swelling was present. The ear was sensitive to touch at the time of the initial exam. The ear was cleaned with hydrogen peroxide and the rabbit was made unavailable for assignment for any practice/testing procedures. On all subsequent exams, the rabbit was behaving normally, eating 100% of the daily feed and there was no sensitivity to touching of the ear. The ear healed uneventfully. This rabbit has been placed in category E because the ear was sensitive to touch indicating pain/distress of a duration that cannot be precisely determined but was likely more than momentary.

1 rabbit #1066 had six days of low feed consumption and mild body weight loss (10.4%) beginning three days after the date of initiation of intravenous dosage practice using saline. The rabbit was used on three separate days over a one week period. The low intake was noted during weekly routine monitoring by the vet staff and the rabbit was immediately removed from practice assignment. The rabbit was bright, alert, responsive and inquisitive. A second water source was provided, daily grooming was started to minimize the loose hair ingested by the rabbit when grooming while feed intake was low, timothy cubes were provided to encourage feed consumption and the pellet diet was provide at two separate locations within the cage. Feed intake improved steadily each day and there was no further weight loss. Treatment was discontinued one week later. This rabbit has been placed in category E acknowledging that distress, likely in response to use for IV practice, was more than momentary since it resulted in six days of low feed intake.

2 rabbits #5408 and #5412 had a 75-100% reduction in feed consumption while used for a five day gavage test. The low intake continued for four additional days after the completion of the testing procedures. Mild body weight loss (10%) also occurred in each rabbit. At examination, both rabbits were bright, alert, responsive and inquisitive. Both rabbits had been provided with an additional water

source, timothy cubes and a rabbit diet with reduced fiber the day prior to the exam. Lab management decided to euthanize these two rabbits on the day of exam to reduce the number of rabbits in the training colony. These rabbits have been placed in category E acknowledging that distress, likely in response to use for gavage testing, was more than momentary since it resulted in nine days of low feed intake prior to euthanasia.

Study: # 51

Animals: 16 Rabbits

Type of Study: 14-Day Oral (Gavage) Toxicity Study of XXX in Rabbits

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis: 16 rabbits from this 14-day general toxicity study conducted in July 2006 through September 2006 have been placed in category E as described in the paragraphs below. The study was approved for 2 male and 2 non-mated female rabbits per group (0, 250, 500 and 1500 mg/kg/day) to dose once daily for 14 days by stomach tube. Expected adverse signs included at approval were soft feces, orange urine, scant feces, weight loss and decreased feed intake. Approval indicated that the 1500 mg/kg/day level was expected to induce toxicity but not death or severe suffering. (In a previous study of this test article in this facility, two rabbits dosed at 600 and 1000 mg/kg/day were euthanized with decreased activity, ptosis and vaginal blood loss after seven daily dosages.) The use of additional water sources and timothy cubes was approved for animals with weight loss. Approval stated that decisions about animals in pain/distress would be made by the study director and vet staff at the time of occurrence. Prior to the start of the study, Amendment 1 was approved for dose administration to be stagger-started with treatment of Groups I and IV occurring prior to Groups II and III. This was to allow review of the 1500 mg/kg/day data for the purpose of selecting dosage levels for Groups II and III. On day 2 of the study, one rabbit in the 1500 MKD level was found dead after dosage administration and the remaining three rabbits in this group were euthanized in poor to moribund condition. As approved by Amendment 2, all rabbits in Group I, II and III were also euthanized on day 2 of the study due to possible exposure to a bacterial infection at the supplier's facility. Rabbits in Groups II and III were never dosed prior to euthanasia. Amendment 2 also extended the study using rabbits from a different supplier and rabbits in the high dosage group (Group IV) received 1000 mg/kg/day. This level resulted in body weight loss, reduced feed consumption and adverse clinical and necropsy signs. As approved by Amendment 3, a dosage level of 1250 mg/kg/day was selected for both Groups II and III. On day 2 of this portion of the study, all rabbits were either found dead or euthanized due to severe adverse signs.

1 rabbit #7197 (male, 1500 MKD) was examined on day 2 of study approximately 2.5 hours after administration of the second dose. The rabbit was prostrate, cold, with no righting reflex, excess salivation and nasal discharge. Euthanasia was recommended but the rabbit died approximately 15 minutes after exam before euthanasia was approved. Adverse signs present at the time of dosage on day 2 included liquid feces and ungroomed coat. Necropsy exam showed erosion/ulceration of the stomach mucosa. This rabbit has been placed in category E because adverse signs that can be associated with pain/distress were present for more than a momentary period prior to death.

1 rabbit #7198 (male, 1500 MKD) was examined on day 2 of study approximately 2.5 hours after administration of the second dose. Pain/distress was diagnosed due to a decreased level of activity and the presence of liquid feces. Adverse signs present at the time of dosage on day 2 and also present at the time of the 2 hour after dose check were ungroomed coat and liquid feces. At re-exam approximately 4.5 hours after dosage, the rabbit was cold and activity remained decreased. Euthanasia was recommended and performed. Necropsy exam showed erosion/ulceration of the stomach. This rabbit has

been placed in category E because adverse signs that can be associated with pain/distress were present for more than a momentary period prior to euthanasia.

2 rabbits #7847 (female, 1500 MKD) and **#7848** (female, 1500 MKD) were examined on day 2 of the study approximately 2.5 hours after administration of the second dose. Pain/distress was diagnosed due to severely decreased activity levels. Adverse signs present for #7847 at the time of dosage on day 2 and also present at the time of the 2 hour after dose check were ungroomed coat and liquid feces. #7848 appeared normal at the time of dosage and ungroomed coat and liquid feces were present at the time of the two hour after dosage check. At re-exam approximately 4.5 hours after dosage, the rabbits were in moribund condition with lack of responsiveness, lack of activity, decreased respiration and hypothermia. Euthanasia was recommended and performed. Necropsy exam showed erosion/ulceration of the stomach for #7848 and no gross lesions were apparent for #7847. These rabbits have been placed in category E because adverse signs that can be associated with pain/distress were present for more than a momentary period prior to euthanasia.

4 rabbits #4499 (male, 1000 MKD), **#4500** (male, 1000 MKD), **#4797** (female, 1000 MKD) and **#4798** (female, 1000 MKD) were dosed for 14 days and euthanized as scheduled on the day after the last dose. Feed intake was reduced to a low to fair level for the entire dosage period and body weight loss ranged from 7-15% over the same period. Timothy cubes were provided daily to each rabbit and #4798 also received a second, moistened timothy cube daily. Adverse signs observed in each of these rabbits during the dosage period included decreased activity, liquid feces, mucoid feces, slow respiration, scant feces, discolored urine, cold to touch, pale extremities, ungroomed coat and urine stained coat. #4798 was examined six times and pain/distress was diagnosed at each exam due to behavior changes and/or to the magnitude of acute body weight loss. At necropsy, #4797 was normal, #4500 and #4499 had tan areas on the liver and #4798 and #4499 had gas distention of the cecum. These rabbits have been placed in category E because adverse signs that can be associated with pain/distress were present for more than a momentary period prior to euthanasia.

8 rabbits #4495 (male, 1250 MKD), **#4496** (male, 1250 MKD), **#4497** (male, 1250 MKD) and **#4795** (female, 1250 MKD) were found dead on study day 2 approximately 2-3 hours after the second dosage. **#4498** (male, 1250 MKD), **#4793** (female, 1250 MKD), **#4794** (female, 1250 MKD), **#4796** (female, 1250 MKD) were euthanized on study day 2 after the second dosage due to adverse signs. Adverse signs seen 2-3 hours after the first dosage included liquid feces and ungroomed coat in all rabbits and impaired righting reflex and decreased activity in #4496. Prior to the second dosage, liquid feces and ungroomed coats were observed. No feed had been consumed overnight and body weight loss in 24 hours ranged from 2.5-7.5%. At the time of the 2-3 hour after dosage check, #4495 and #4497 were found dead and each of the remaining six rabbits showed adverse signs including prostration, decreased activity, impaired righting reflex, slow respiration and cold to touch. #4496 and #4795 were found dead approximately 30 minutes later and the remaining four rabbits were euthanized by study director recommendation later that afternoon. All rabbits had red tracheal linings and/or stomach erosions/ulcerations except for #4498 with a fluid-filled lung cyst and #4794 with no adverse findings. These rabbits have been placed in category E because adverse clinical signs and/or adverse necropsy signs that can be associated with pain/distress were present for more than a momentary period prior to death or euthanasia.

CHARLES RIVER LABS PRECLINICAL SERVICES - ARKANSAS (Redfield, AK - 016)

Study: #52

Animals: 1 Dog

Type of Study: Dose Range-Finding Study of a Test Article administered Orally (Gavage)

Guidelines/Regulations:

- Although, this study was conducted non-GLP, this range-finding study was to determine dose levels for a 28-day study, which will be necessary for IND submission to FDA. Meeting minutes to the Pre-IND meeting between the Sponsor and FDA were supplied to Charles River Laboratories with specific preclinical information needed for IND submission.

Diagnosis: 1 female dog #2006

- At 5 mg/kg and between Study Days 1 and 3, there were no abnormal findings.
- At 10 mg/kg and between Study Days 4 and 7, vomiting (Study Days 4 through 6) and abnormal stool (Study Days 5 and 6) were recorded.
- At 20 mg/kg and on Study Day 8, vomiting, labored breathing, decreased activity, and increased salivation were recorded prior to the animal being found dead at the 1 hour postdose electrocardiogram measurement.
- Acute signs and symptoms were not definitive of moribundity.
- The death of the animal was acute (animal found dead); therefore, euthanasia could not take place.

Study: #53

Animals: 1 Dog

Type of Study: Exploratory Toxicity Study of a Test Article Administered by Intravenous Infusion

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis: 1 male dog #14349

- Abnormal clinical observations for this dog progressed from pale gums to slow capillary refill time during the infusion to decreased activity, paling of gums, decreased respiratory rate, labored breathing, and non-arousal at 5 minutes post-infusion.
- The progression of adverse signs and death was acute and euthanasia procedures could not be implemented in time to relieve the pain and/or distress.

Study: #54

Animals: 1 Dog (mid-dose)

Type of Study: A 10-Day Toxicity Study of a Test Article Administered by Oral Gavage

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis:

1 dog #11771 was found dead on Study 3 after observing clinical signs of vomitus and increased salivation on Study Days 1 and 2. On Study Day 2, this animal also had observations of ataxia, decreased activity and abnormal stool. Supportive care consisting of food and water supplements and fluid strategies to maintain the hydration and nutritional level of the animal were provided. The death was unexpected.

Study: #55

Animals: 1 Dog (mid-dose)

Type of Study: A 10-Day Toxicity Study of a Test Article Administered by Oral Gavage

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis:

1 dog #11773 was observed with clinical signs of vomitus and increased salivation on Study Days 1 through 4, before euthanized in moribund condition on Study Day 5. Supportive care of food and water supplements as well as fluid strategies had been put into place to maintain the health of the animal. The dog was euthanized in moribund condition, however it is possible that the clinical signs prior to euthanasia resulted in some unalleviated pain or distress that was more than momentary in duration.

Study: #56

Animals: 1 Dog (high-dose)

Type of Study: A 10-Day Toxicity Study of a Test Article Administered by Oral Gavage

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis:

1 dog #11777 was observed with clinical signs vomitus and increased salivation, activity decreased, and loose skin for up to 5 days prior to being euthanized in moribund condition on Study Day 7. Supportive care of food and water supplements as well as fluid strategies had been put into place to maintain the health of the animal. The dog was euthanized in moribund condition, however it is possible that the clinical signs prior to euthanasia resulted in some unalleviated pain or distress that was more than momentary in duration.

Study: #57

Animals: 1 Dog (dosed at the 10/30/100/300 mg/kg levels)

Type of Study: Escalating Dose Toxicity Study of a test Article Administered by Oral Gavage

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis:

1 female dog #13270 died 1 day following the dose at 300 mg/kg on Study Day 11. Observations recorded prior to death included decreased activity, labored breathing, and impaired righting reflex. Decreased activity and labored breathing were also observed at the 10/30/100 mg/kg dose levels, in most dogs on study, but were of a short duration (all animals were normal at 2 hours post-dose). The Study Director was aware of the dog's condition and had planned on euthanasia after the 24-hour blood collection. This dog died during the 24-hour blood collection. Hemoperitoneum was considered to be the cause of death at necropsy.

Study: #58

Animals: 12 Dogs (all dose levels)

Type of Study: Oral Toxicity and Pharmacokinetic Study

Guidelines/Regulations:

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis:

12 dogs was observed with clinical signs of vomitus, watery or soft stool, and increased salivation seen during the first 4 days of the study. Due to excessive vomiting seen post-dose on these days, the animals were given a washout for 3 days followed by dose administration once daily for 14 additional days at lower dosage levels and volumes. After the dosages and volumes were lowered, sporadic vomitus, abnormal stool, increased salivation, and/or red skin discoloration of the ears were seen at all dosage levels tested. There were no other signs of toxicity noted during the study and there were no abnormal histopathology findings. Although these signs would not generally be considered painful, the Study Director believed the duration of the signs could have been distressful.

Study: #59

Animals: 9 Pigs (all dose levels)

Type of Study: Escalating Dose Toxicology Study of Intravenously Administered Test Article

Guidelines/Regulations:

- The U.S. Food and Drug Administration required that animal toxicology studies be performed in a rodent animal species and a non-rodent animal species. Although this study was conducted non-GLP, a non-rodent species study was conducted to meet the FDA requirements.
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance for Industry, MD(R1) Non-clinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals

Diagnosis:

9 pigs among Groups 3 through 5 (low-, mid-, and high-dose) were observed with the following clinical signs: increased salivation, excessive lacrimation, vomitus and labored breathing. These pigs were also observed with clinical signs indicative of local inflammatory reaction at and around the site of injection (swelling, necrosis). 1 pig in the low-dose group had tremors of a forelimb which lasted one day. 1 pig in the mid-dose group had tremors and a forefoot which lasted three days. All of these effects were transient and generally lasted 1-3 days. The study was designed to determine if the effects were transient. All animals survived to the scheduled terminal euthanasia. There were no remarkable test article-related findings associated with body weights, clinical pathology, organ weights, as well as macroscopic or microscopic examinations. These animals were categorized as E because the clinical signs observed may be consistent with distress that was more than momentary in duration.

Study: #60

Animals: 2 Primates (high-dose)

Type of Study: A Rising dose Tolerance and 10-Day Pilot Toxicity Study of an Anti-Cancer Agent

Guidelines/Regulations:

- U.S. Food and Drug Administration (2005) Investigational New Drug Application, Title 21, Part 321.23, 8.ii.a

Diagnosis:

2 primates #XXX00027 and XXX40131X - At 3 hours post-dosing the animals were vomiting. At 6 hours animals were eating, active and appeared clinically normal. Post-dosing observations were scheduled at hourly observations for the first 3 hours. At the last observation the animals did not appear to need clinical intervention. Animals were found dead in the cage in the morning. A moderate amount of watery feces was found in the cage pan. Gross necropsy revealed a bright red and swollen colon in these animals. While the animals appeared clinically normal at the last observation prior to being found dead, it is possible based on the gross necropsy results that the animals may have experienced more than momentary pain or distress prior to dying.

Study: #61

Animals: 1 Primate

Type of Study: General Toxicity Study. An 8-month Repeat Dose Prostrate Injection Safety Study in Primates with a 6-month Recovery Period

Guidelines/Regulations:

- U.S. Food and Drug Administration (2005) Investigational New Drug Application, Title 21, Part 321.23, 8.ii.a

Diagnosis:

1 primate #XXX00064 - Animal had a hunched appearance in the cage and a history of bladder stones. It is possible that this was related to test material. Clinical intervention in the form of attempted bladder catheterization was performed however the urethra was blocked. In addition analgesics and anesthesia was given at the time of the finding. It was determined that the bladder had ruptured and the animal was euthanized. The animal is categorized as E as it is possible that the animal experienced more than momentary distress prior to clinical intervention.

IACUC-APPROVED EXCEPTIONS TO REGULATIONS AND STANDARDS

The IACUC and the Attending Veterinarian must approve exemptions from non-human primate environmental enhancement plans and dog exercise activities. Monitoring of these animals during the period that they are exempted is performed by the technical staff with oversight from the Veterinary Staff to ensure that the animals do not exhibit stereotypic behaviors that are detrimental to their overall health and well-being. The following exceptions to standards/regulations were approved by the IACUC during this reporting period.

Species: Macaca sp. (Cynomolgus and/or Rhesus)
Number: 347

All animals were on metabolism and pharmacokinetics studies and may have been used on more than one study. Pair housing, environmental enrichment devices inside the cage and/or dietary restrictions (no fruit peels or peanut shells) were withheld after dose administration for 1-15 days during sample collection. Environmental enrichment devices were allowed outside the cage. External stimuli such as radios, televisions, and conspecific visualization, olfactory and auditory stimulation were provided.

Species: Macaca sp. (Cynomolgus)
Number: 168

Exemption: 118 cynos were exempted for 2-29 days, 18 cynos were exempted 50 days and 32 cynos exempted 176 days.

All animals were on toxicology studies. Pair housing was exempted after dose administration, as defined above, during sample collection. Environmental enrichment devices and external stimuli such as radios, televisions, and conspecific visualization, olfactory and auditory stimulation were provided.

Species: Macaca sp. (Cynomolgus)
Number: 64

Animals were on pharmacology or surgical studies. Animals on pharmacology studies may have been used on more than one study. Pair housing was exempted post-surgery to facilitate recovery. Pair housing was exempted after dose administration for up to 15 days during sample collection. Environmental enrichment devices and external stimuli such as radios, televisions, and conspecific visualization, olfactory and auditory stimulation were provided.

Species: Canis familiaris (Dog)
Number: 261

All animals were on metabolism and pharmacokinetics studies and may have been used on more than one study. Pair housing and/or exercise was withheld after dose administration for 1-15 days during sample collection. The square footage of the caging met all requirements for housing the animals, however, it did not meet the additional space needs to eliminate the requirement for exercise outside of the cage. The animals were observed daily for signs of distress from lack of exercise during the exemption period. No animals were noted to be adversely affected during this period.

Species: Canis familiaris (Dog)
Number: 81

Exemption: 34 dogs were exempted for 15 days, 12 dogs were exempted 42 days and 35 dogs were exempted 90-98 days.

All animals were on toxicology studies. Pair housing and/or exercise were exempted during dose administration and sample collection. The square footage of the caging met all requirements for housing the

animals, however, it did not meet the additional space needs to eliminate the requirement for exercise outside of the cage. The animals were observed daily for signs of distress from lack of exercise during the exemption period. No animals were noted to be adversely affected during this period.

Species: Canis familiaris (Dog)

Number: 80

Animals were on pharmacology or surgical studies. Animals on pharmacology studies may have been used on more than one study. Pair housing was exempted post-surgery to facilitate recovery. Pair housing and/or exercise was exempted after dose administration for up to 15 days during sample collection or post-surgery for up to 35 days to facilitate recovery. The square footage of the caging met all requirements for housing the animals, however, it did not meet the additional space needs to eliminate the requirement for exercise outside of the cage. The animals were observed daily for signs of distress from lack of exercise during the exemption period. No animals were noted to be adversely affected during this period.